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## PRIORITY DOCUMENT

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Modtaget

## TITLE: Improved Bacillus Host Cell

#### **TECHNICAL FIELD**

Bacillus sp. are attractive hosts for the production of heterologous proteins due their ability to secrete proteins directly into the culture medium. They have a high capacity for protein secretion, are genetically highly amenable, nonpathogenic and free of endotoxins, and consequently a large variety of proteins from different organisms have been efficiently produced and secreted in Bacillus sp. i.e. in Bacillus licheniformis.

In the highly competitive biotech industry, even slightly improved *Bacillus* host cells are in demand, which may provide more attractive production systems, or may even just be alternative production systems.

#### BACKGROUND

Many industrial products of commercial interest can be produced biologically in *Bacillus sp.* host cells e.g. heterologous polypeptides, amino acids, carbohydrates etc. Some of these products are sold as process aids, intermediates, or even end-products in the food and feed industries as well as in the pharmaceutical industry. There are increasingly strict regulations that must be complied with when producing such products in microbial production hosts for sale in these industries, for instance the presence of bacterial spores in the products is seen as a problem. When producing in *Bacillus licheniformis* it is thus desirable to ensure that the host cell is not capable of forming spores.

#### SUMMARY

A problem to be solved by the present invention is how to obtain a *Bacillus licheniformis* host cell incapable of forming spores, or how to impair the sporulation process of said cell. The present invention provides a solution to the problem by providing a *Bacillus licheniformis* host cell which has a reduced capacity to produce one or more polypeptide(s) involved in sporulation.

Accordingly, in a first aspect the invention relates to a *Bacillus licheniformis* mutant host cell derived from a parent *B. licheniformis* host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, preferably at least 85% identical, more preferably at least 90% identical, still more preferably at least 95% identical, and most preferably at least 97% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions. Preferably the mutant host cell expresses at

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least 10% less, more preferably at least 20% less, still more preferably at least 30% less, even more preferably at least 40% less, yet more preferably at least 50% less, or at least 60% less, or at least 80%, or most preferably at least 90% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions. Most preferably the mutant host cell expresses absolutely nothing of the one or more polypeptide(s) involved in sporulation.

Comparable conditions of cultivation must be used in order to compare the expression level of the one or more polypeptide(s) involved in sporulation in a mutant host cell of the invention with that in a parent host cell. They are cultivated separately under identical conditions in identical setups, of course allowing for the usual standard deviations of the operating parameters normally associated with growth experiments, such as temperature control etc. The quantification of the expression level of the one or more polypeptide(s) is done by standard text-book assay techniques as known in the art e.g. mRNA quantification or immuno-based assays.

In a second aspect the invention relates to a process for producing at least **one** product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in the previous aspect in a suitable medium, whereby the said product is produced.

Finally, an aspect of the invention relates to a use of a *Bacillus licheniformis* mutant host cell as defined in the first aspect for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced.

#### **DEFINITIONS**

Nucleic acid construct: When used herein, the term "nucleic acid construct" means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or which has been modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature. The term nucleic acid construct is synonymous with the term "expression cassette" when the nucleic acid construct contains the control sequences required for expression of a coding sequence of the present invention.

Control sequence: The term "control sequences" is defined herein to include all components, which are necessary or advantageous for the expression of a polypeptide of the present invention. Each control sequence may be native or foreign to the nucleotide sequence encoding the polypeptide. Such control sequences include, but are not limited to, a leader,

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polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. At a minimum, the control sequences include a promoter, and transcriptional and translational stop signals. The control sequences may be provided with linkers for the purpose of introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the nucleotide sequence encoding a polypeptide.

Operably linked: The term "operably linked" is defined herein as a configuration in which a control sequence is appropriately placed at a position relative to the coding sequence of the DNA sequence such that the control sequence directs the expression of a polypeptide.

Coding sequence: When used herein the term "coding sequence" is intended to cover a nucleotide sequence, which directly specifies the amino acid sequence of its protein product. The boundaries of the coding sequence are generally determined by an open reading frame, which usually begins with the ATG start codon. The coding sequence typically include DNA, cDNA, and recombinant nucleotide sequences.

Expression: In the present context, the term "expression" includes any step involved in the production of the polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

Expression vector: In the present context, the term "expression vector" covers a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of the invention, and which is operably linked to additional segments that provide for its transcription.

### **DETAILED DISCLOSURE**

A Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions.

The term "parent host cell" in the context of the present invention means a cell which is genetically identical, or isogenic, to the progeny mutant or mutant cell of the present invention, except for the mutated one or more gene(s) encoding one or more polypeptide(s) involved in sporulation in said mutant.

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The degree of identity, or %-identity of polypeptide sequences can suitably be investigated by aligning the sequences using a computer program known in the art, such as "GAP" provided in the GCG program package (Program Manual for the Wisconsin Package, Version 8, August 1994, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711)(Needleman, S.B. and Wunsch, C.D., (1970), Journal of Molecular Biology, 48, 443–453). Using GAP with the following settings for DNA sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3".

An object of the present invention is to provide a culture medium free of bacterial spores so as to reduce the product purification to a minimum, and to comply with regulatory requirements. This may be done according to the invention by reducing or even completely abolishing the expression of one or more gene(s) encoding a native polypeptide(s) involved in sporulation via mutagenisation of that (those) gene(s). One of the very well-known method of ensuring that a gene is not expressed into an active polypeptide within a cell is simply to delete or partially delete the encoding gene. Many techniques have been described in the art on how to specifically delete or partially delete one or more gene(s) in the genome of a cell, and certainly from the genome of a *Bacillus licheniformis* cell (see e.g. Novozymes A/S WO 01/90393, Novozymes A/S WO 02/00907). Accordingly, a preferred embodiment of the present invention relates to a host cell of the first aspect, which is mutated by a partial or complete deletion of the one or more gene(s) encoding the one or more polypeptide(s) involved in sporulation.

A preferred embodiment of the present invention relates to a host cell of the first aspect, which is mutated in two or more genes encoding two or more polypeptides involved in sporulation.

The product of interest to be produced by the mutant host cell of the first aspect may be one or more polypeptide(s) encoded by one or more heterologous gene(s). Consequently, a preferred embodiment of the present invention relates to a host cell of the first aspect, which comprises one or more heterologous gene(s) encoding one or more heterologous polypeptide(s).

In the industrial production of polypeptides it is of interest to achieve a product yield as high as possible. One way to increase the yield is to increase the copy number of a gene encoding a polypeptide of interest. This can be done by placing the gene on a high copy number plasmid. However, plasmids are unstable and are often lost from the host cells if

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there is no selective pressure during the cultivation of the host cells. Another way to increase the copy number of the gene of interest is to integrate it into the host cell chromosome in multiple copies. Integration of two genes has been described in WO 91/09129 and WO 94/14968 (Novozymes A/S) the content of which is hereby incorporated by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) is present in at least two copies, preferably at least 4 copies, and most preferably at least 6 copies. In another embodiment the heterologous gene(s) is present in at least ten copies. If carried on a plasmid the gene(s) may be present in several hundred copies per cell, so in a still further embodiment of the present invention the heterologous gene(s) is present in at least 100 copies.

Integration of two genes closely spaced in anti-parallel tandem to achieve better stability has been described in WO 99/41358 (Novozymes A/S) the content of which is hereby incorporated by reference, as well as the stable chromosomal multi-copy integration of genes described in WO 02/00907 (Novozymes A/S) the content of which is incorporated herein by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are stably integrated into the genome of the cell.

Selection of chromosomal integrant has for convenience resulted in the use of selectable markers such as antibiotic resistance markers. However it is desirable if possible to avoid the use of antibiotic marker genes. WO 01/90393 discloses a method for the integration of a gene in the chromosome of a host cell without leaving antibiotic resistance markers behind in the strain, the content of which is hereby incorporated by reference A preferred embodiment of the present invention relates to a host cell of the first aspect wherein the heterologous gene(s) is integrated into the genome of the cell without leaving any antibiotic resistance marker gene(s) at the site of integration.

The present invention also relates to nucleic acid constructs comprising a nucleotide sequence encoding a product of interest, which may be operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

A nucleotide sequence encoding a polypeptide ofinterest may be manipulated in a variety of ways to provide for expression of the polypeptide. Manipulation of the nucleotide sequence prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying nucleotide sequences utilizing recombinant DNA methods are well known in the art.

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Other ways of increasing the product yield would be to increase promoter activity of the specific promoter regulating the expression of a specific gene of interest. Also a more general increase in the activity of several promoters at the same time could lead to an improved product yield. The control sequence may be an appropriate promoter sequence, a nucleotide sequence which is recognized by a host cell for expression of the nucleotide sequence. The promoter sequence contains transcriptional control sequences, which mediate the expression of the polypeptide. The promoter may be any nucleotide sequence which shows transcriptional activity in the host cell of choice including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

Examples of suitable promoters for directing the transcription of the nucleic acid constructs of the present invention, especially in a bacterial host cell, are the promoters obtained from the E. coli lac operon, Streptomyces coelicolor agarase gene (dagA), Bacillus subtilis levansucrase gene (sacB), Bacillus licheniformis alpha-amylase gene (amyL), Bacillus stearothermophilus maltogenic amylase gene (amyM), Bacillus amyloliquefaciens alpha-amylase gene (amyQ), Bacillus licheniformis penicillinase gene (penP), Bacillus subtilis xylA and xylB genes, and prokaryotic beta-lactamase gene (Villa-Kamaroff et al., 1978, Proceedings of the National Academy of Sciences USA 75: 3727-3731), as well as the tac promoter (DeBoer et al., 1983, Proceedings of the National Academy of Sciences USA 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242: 74-94; and in Sambrook et al., 1989, supra.

Other useful promoters are described in WO 93/10249, WO 98/07846, and WO 99/43835 (Novozymes A/S) the contents of which are incorporated fully herein by reference. A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are transcribed from a heterologous promoter or from an artificial promoter.

The control sequence may also be a suitable transcription terminator sequence, a sequence recognized by a host cell to terminate transcription. The terminator sequence is operably linked to the 3' terminus of the nucleotide sequence encoding the polypeptide. Any terminator which is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a suitable leader sequence, a nontranslated region of an mRNA which is important for translation by the host cell. The leader sequence is operably

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linked to the 5' terminus of the nucleotide sequence encoding the polypeptide. Any leader sequence that is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a polyadenylation sequence, a sequence operably linked to the 3' terminus of the nucleotide sequence and which, when transcribed, is recognized by the host cell as a signal to add polyadenosine residues to transcribed mRNA. Any polyadenylation sequence which is functional in the host cell of choice may be used in the present invention.

The control sequence may also be a signal peptide coding region that codes for an amino acid sequence linked to the amino terminus of a polypeptide and directs the encoded polypeptide into the cell's secretory pathway. The 5' end of the coding sequence of the nucleotide sequence may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted polypeptide. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region which is foreign to the coding sequence. The foreign signal peptide coding region may be required where the coding sequence does not naturally contain a signal peptide coding region. Alternatively, the foreign signal peptide coding region may simply replace the natural signal peptide coding region in order to enhance secretion of the polypeptide. However, any signal peptide coding region which directs the expressed polypeptide into the secretory pathway of a host cell of choice may be used in the present invention.

Effective signal peptide coding regions for bacterial host cells are the signal peptide coding regions obtained from the genes for Bacillus NCIB 11837 maltogenic amylase, Bacillus stearothermophilus alpha-amylase, Bacillus licheniformis subtilisin, Bacillus licheniformis beta-lactamase, Bacillus stearothermophilus neutral proteases (nprT, nprS, nprM), and Bacillus subtilis prsA. Further signal peptides are described by Simonen and Palva, 1993, Microbiological Reviews 57: 109-137.

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The control sequence may also be a propeptide coding region that codes for an amino acid sequence positioned at the amino terminus of a polypeptide. The resultant polypeptide is known as a proenzyme or propolypeptide (or a zymogen in some cases). A propolypeptide is generally inactive and can be converted to a mature active polypeptide by catalytic or autocatalytic cleavage of the propeptide from the propolypeptide. The propeptide coding region may be obtained from the genes for Bacillus subtilis alkaline protease (aprE), Bacillus

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subtilis neutral protease (nprT), Saccharomyces cerevisiae alpha-factor, Rhizomucor miehei aspartic proteinase, and Myceliophthora thermophila laccase (WO 95/33836).

Where both signal peptide and propertide regions are present at the amino terminus of a polypeptide, the propertide region is positioned next to the amino terminus of a polypeptide and the signal peptide region is positioned next to the amino terminus of the propertide region.

It may also be desirable to add regulatory sequences which allow the regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory systems are those which cause the expression of the gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Regulatory systems in prokaryotic systems include the lac, tac, and trp operator systems. In yeast, the ADH2 system or GAL1 system may be used. In eukaryotic systems, these include the dihydrofolate reductase gene which is amplified in the presence of methotrexate, and the metallothionein genes which are amplified with heavy metals. In these cases, the nucleotide sequence encoding the polypeptide would be operably linked with the regulatory sequence.

The present invention also relates to recombinant expression vectors comprising the nucleic acid construct of the invention. The various nucleotide and control sequences described above may be joined together to produce a recombinant expression vector which may include one or more convenient restriction sites to allow for insertion or substitution of the nucleotide sequence encoding the polypeptide at such sites. Alternatively, the nucleotide sequence of the present invention may be expressed by inserting the nucleotide sequence or a nucleic acid construct comprising the sequence into an appropriate vector for expression. In creating the expression vector, the coding sequence is located in the vector so that the coding sequence is operably linked with the appropriate control sequences for expression.

The recombinant expression vector may be any vector (e.g., a plasmid or virus) which can be conveniently subjected to recombinant DNA procedures and can bring about the expression of the nucleotide sequence. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vectors may be linear or closed circular plasmids.

The vector may be an autonomously replicating vector, i.e., a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication.

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e.g., a plasmid, an extrachromosomal element, a minichromosome, or an artificial chromosome.

The vector may contain any means for assuring self-replication. Alternatively, the vector may be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Furthermore, a single vector or plasmid or two or more vectors or plasmids which together contain the total DNA to be introduced into the genome of the host cell, or a transposon may be used.

The vectors of the present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like.

Examples of bacterial selectable markers are the dal genes from Bacillus subtilis or Bacillus licheniformis, or markers which confer antibiotic resistance such as ampicillin, kanamycin, chloramphenicol or tetracycline resistance.

The vectors of the present invention preferably contain an element(s) that permits stable integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent of the genome.

For integration into the host cell genome, the vector may rely on the nucleotide sequence encoding the polypeptide or any other element of the vector for stable integration of the vector into the genome by homologous or nonhomologous recombination. Alternatively, the vector may contain additional nucleotide sequences for directing integration by homologous recombination into the genome of the host cell. The additional nucleotide sequences enable the vector to be integrated into the host cell genome at a precise location(s) in the chromosome(s). To increase the likelihood of integration at a precise location, the integrational elements should preferably contain a sufficient number of nucleotides, such as 100 to 1,500 base pairs, preferably 400 to 1,500 base pairs, and most preferably 800 to 1,500 base pairs, which are highly homologous with the corresponding target sequence to enhance the probability of homologous recombination. The integrational elements may be any sequence that is homologous with the target sequence in the genome of the host cell. Furthermore, the integrational elements may be non-encoding or encoding nucleotide sequences. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

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For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. Examples of bacterial origins of replication are the origins of replication of plasmids pBR322, pUC19, pACYC177, and pACYC184 permitting replication in E. coli, and pUB110, pE194, pTA1060, and pAMß1 permitting replication in Bacillus. The origin of replication may be one having a mutation which makes its functioning temperature-sensitive in the host cell (see, e.g., Ehrlich, 1978, Proceedings of the National Academy of Sciences USA 75: 1433).

More than one copy of a nucleotide sequence of the present invention may be inserted into the host cell to increase production of the gene product. An increase in the copy number of the nucleotide sequence can be obtained by integrating at least one additional copy of the sequence into the host cell genome or by including an amplifiable selectable marker gene with the nucleotide sequence where cells containing amplified copies of the selectable marker gene, and thereby additional copies of the nucleotide sequence, can be selected for by cultivating the cells in the presence of the appropriate selectable agent.

The procedures used to ligate the elements described above to construct the recombinant expression vectors of the present invention are well known to one skilled in the art (see, e.g., Sambrook et al., 1989, supra).

The introduction of a vector into a bacterial host cell may, for instance, be effected by protoplast transformation (see, e.g., Chang and Cohen, 1979, Molecular General Genetics 168: 111-115), using competent cells (see, e.g., Young and Spizizin, 1961, Journal of Bacteriology 81: 823-829, or Dubnau and Davidoff-Abelson, 1971, Journal of Molecular Biology 56: 209-221), electroporation (see, e.g., Shigekawa and Dower, 1988, Biotechniques 6: 742-751), or conjugation (see, e.g., Koehler and Thorne, 1987, Journal of Bacteriology 169: 5771-5278).

A preferred embodiment of the present invention relates to a host cell of the first aspect, wherein the heterologous gene(s) are comprised in an operon, preferably a polycistronic operon. The term "operon" in the context of the present invention means a polynucleotide comprising several genes that are clustered and perhaps even transcribed together into a polycistronic mRNA, e.g. genes coding for the enzymes of a metabolic pathway. The transcription of an operon may be initiated at a promoter region and controlled by a neighboring regulatory gene, which encodes a regulatory protein, which in turn binds to the operator sequence in the operon to respectively inhibit or enhance the transcription. The

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gene or the operon can be carried on a suitable plasmid that can be stably maintained, e.g. capable of stable autonomous replication in the host cell (the choice of plasmid will typically depend on the compatibility of the plasmid with the host cell into which the plasmid is to be introduced) or it can be carried on the chromosome of the host. The said gene may be endogenous to the host cell in which case the product of interest is a protein naturally produced by the host cell and in most cases the gene will be in it normal position on the chromosome. If the gene encoding the product of interest is an exogenous gene, the gene could either be carried on a suitable plasmid or it could be integrated on the host chromosome. In one embodiment of the invention the eubacterium is a recombinant eubacterium. Also the product of interest may in another embodiment be a recombinant protein.

The product of interest is any gene product or product of a metabolic pathway which is industrially useful and which can be produced in a bacterial cell such as a *B. licheniformis*.

In one preferred embodiment, the heterologous polypeptide(s) is an antimicrobial peptide, or a fusion peptide comprising a peptide part which in its native form has antimicrobial activity.

In another preferred embodiment, the heterologous polypeptide(s) has biosynthetic activity and produces a compound or an intermediate of interest.

Yet another embodiment relates to a host cell of the first aspect, wherein the compound or intermediate of interest comprises vitamins, amino acids, antibiotics, carbohydrates, or surfactants, and preferably the carbohydrates comprise hyaluronic acid.

In one embodiment the heterologous polypeptide(s) is an enzyme, particularly the enzyme is an enzyme of a class selected from the group of enzyme classes consisting of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5), and ligases (EC 6). Preferably the enzyme is an enzyme with an activity selected from the group consisting of aminopeptidase, amylase, amyloglucosidase, mannanase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, polyphenoloxidase, phytase, phenoloxidase, protease, ribonuclease, transferase, transglutaminase, or xylanase. Preferably the enzyme is an amylase or a mannanase.

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A second aspect of the invention relates to a process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in the first aspect of the invention in a suitable medium, whereby the said product is produced. One embodiment relates to a process of the second aspect, further comprising isolating or purifying the product of interest. Suitable media for the cultivation is described below as well as methods for the purification or isolation of the produced product which is an optional additional step to the process of the present invention.

In the production methods of the present invention, the cells are cultivated in a nutrient medium suitable for production of the polypeptide using methods known in the art. For example, the cell may be cultivated by shake flask cultivation, small-scale or large-scale fermentation (including continuous, batch, fed-batch, or solid state fermentations) in laboratory or industrial fermentors performed in a suitable medium and under conditions allowing the polypeptide to be expressed and/or isolated. The cultivation takes place in a suitable nutrient medium comprising carbon and nitrogen sources and inorganic salts, using procedures known in the art. Suitable media are available from commercial suppliers or may be prepared according to published compositions (e.g., in catalogues of the American Type Culture Collection). If the polypeptide is secreted into the nutrient medium, the polypeptide can be recovered directly from the medium. If the polypeptide is not secreted, it can be recovered from cell lysates.

The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The media are prepared using procedures known in the art (see, e.g., references for bacteria and yeast; Bennett, J.W. and LaSure, L., editors, *More Gene Manipulations in Fungi*, Academic Press, CA, 1991).

The polypeptides may be detected using methods known in the art that are specific for the polypeptides. These detection methods may include use of specific antibodies, formation of an enzyme product, or disappearance of an enzyme substrate. For example, an enzyme assay may be used to determine the activity of the polypeptide as described herein.

35 The resulting polypeptide may be recovered by methods known in the art. For example, the polypeptide may be recovered from the nutrient medium by conventional procedures

including, but not limited to, centrifugation, filtration, extraction, spray-drying, evaporation, or precipitation.

The polypeptides of the present invention may be purified by a variety of procedures known in the art including, but not limited to, chromatography (e.g., ion exchange, affinity, hydrophobic, chromatofocusing, and size exclusion), electrophoretic procedures (e.g., preparative isoelectric focusing), differential solubility (e.g., ammonium sulfate precipitation), SDS-PAGE, or extraction (see, e.g., Protein Purification, J.-C. Janson and Lars Ryden, editors, VCH Publishers, New York, 1989).

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A third aspect of the present invention relates to the use of a *Bacillus licheniformis* mutant host cell as defined in the first aspect for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced, and optionally isolating or purifying the produced product.

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#### CLAIMS

- 1. A Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions.
- 2. The host cell according to claim 1, which is mutated by a partial or complete deletion of the one or more gene(s) encoding the one or more polypeptide(s) involved in sporulation.
  - 3. The host cell according to any of claims 1 2, which is mutated in two or more genes encoding two or more polypeptides involved in sporulation.
- 5. The host cell according to any of claims 1 4, which comprises one or more heterologous gene(s) encoding one or more heterologous polypeptide(s).
  - 6. The host cell according to claim 5, wherein the heterologous gene(s) is present in at least two copies.
  - 7. The host cell according to claim 5 or 6, wherein the heterologous gene(s) are stably integrated into the genome of the cell.
- 8. The host cell according to any of claims 5 7, wherein the heterologous gene(s) is integrated into the genome of the cell without leaving any antibiotic resistance marker genes at the site of integration.
  - 9. The host cell according to any of claims 5 8, wherein the heterologous gene(s) are transcribed from a heterologous promoter or from an artificial promoter.
  - 10. The host cell according to any of claim 5-9, wherein the heterologous gene(s) are comprised in an operon, preferably a polycistronic operon.
- 11. The host cell according to any of claims 5 10, wherein the heterologous polypeptide(s)
  is an antimicrobial peptide, or a fusion peptide comprising a peptide part which in its native form has antimicrobial activity.

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- 12. The host cell according to any of claims 5 10, wherein the heterologous polypeptide(s) has biosynthetic activity and produces a compound or an intermediate of interest.
- 13. The host cell according to claim 12, wherein the compound or intermediate of interest comprises vitamins, amino acids, antibiotics, carbohydrates, or surfactants.
- 14. The host cell according to claim 13, wherein the carbohydrates comprise hyaluronic acid.
- 15. The host cell according to any of claims 5 10, wherein the heterologous polypeptide(s) is an enzyme, preferably a secreted enzyme.
  - 16. The host cell according to claim 15, wherein the enzyme is is an enzyme of a class selected from the group of enzyme classes consisting of oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3), lyases (EC 4), isomerases (EC 5), and ligases (EC 6).
  - 17. The host cell according to claim 16, wherein the enzyme is an enzyme with an activity selected from the group of enzyme activities consisting of aminopeptidase, amylase, amyloglucosidase, mannanase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, galactosidase, beta-galactosidase, glucoamylase, glucose oxidase, glucosidase, haloperoxidase, hemicellulase, invertase, isomerase, laccase, ligase, lipase, lyase, mannosidase, oxidase, pectinase, peroxidase, phytase, phenoloxidase, polyphenoloxidase, protease, ribonuclease, transferase, transglutaminase, and xylanase.
  - 18. The host cell according to claim 17, wherein the enzyme is an amylase or a mannanase.
  - 19. A process for producing at least one product of interest in a *Bacillus licheniformis* mutant host cell, comprising cultivating a *B.licheniformis* mutant host cell as defined in any of the claims 1 18 in a suitable medium, whereby the said product is produced.
  - 20. The process according to claim 19, further comprising isolating or purifying the product of interest.
- 21. A use of a Bacillus licheniformis mutant host cell as definde in any of the claims 1 18 for producing at least one product of interest comprising cultivating the mutant host cell in a suitable medium whereby the said product is produced.

22. The use according to claim 21 further comprising isolating or purifying the product of interest.

#### **ABSTRACT**

TITLE: Improved Bacillus Host Cell.

A Bacillus licheniformis mutant host cell derived from a parent B. licheniformis host cell, which mutant host cell is mutated in one or more gene(s) encoding one or more polypeptide(s) involved in sporulation which is at least 80% identical to one or more of the polypeptides shown in SEQ ID NO's: 2 to 129, wherein the mutant host cell expresses at least 5% less of the one or more polypeptide(s) involved in sporulation than the parent host cell, when they are cultivated under comparable conditions.

Patent- og Varemærkestyrelsen 1 0 APR, 2002

Modtaget

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Jørgensen, Steen Troels

Olesen, Peter Bjarke

Andersen, Jens Tønne

Rasmussen, Michael Dolberg

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Bacillus licheniformis

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tta aga gag ctt ctt Leu Arg Glu Leu Leu 55

677

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gac ttg tat ttt gat gcg ggt gat ctg ttg gtg ctg gcg aaa acg ac Asp Leu Tyr Phe Asp Ala Gly Asp Leu Leu Val Leu Ala Lys Thr The 80 85 90	
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agg aga gtg ctt gtt aaa caa atc aat gcg gcc att aag ttg cat ga Arg Arg Val Leu Val Lys Gln Ile Asn Ala Ala Ile Lys Leu His Gl 110 115 120	a 869 u
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Lys Lys Ile Ser Asp Pro Gln Leu Arg Gln Leu Tyr Ser Val Ser Ala 35 40 45 Page 13

Lys Ala Leu Glu Gln Asn Leu Arg Glu Leu Leu Pro Phe Leu Pro Lys 50												
Ala Pro Ala Phe Gln Arg Glu Asp Glu Arg Ala Asp Leu Tyr Phe Asp 65 70 75 80												
Ala Gly Asp Leu Leu Val Leu Ala Lys Thr Thr Val Arg Asn Tyr Ala 85 90 95												
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ago ser	cgg Arg 305	Leu	tcg Ser	cgg Arg	tat Tyr	gcg Ala 310	· Va I	aac Asn	1 Trp	cac His	315	i ini	atc Tle	gcg Ala	cct Pro	1139

Page 15

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Gln Tyr Pro Asp Asn Lys Glu Lys Ala Ala Phe His Leu Asn Ala Glu 65 70 75 80	
Tyr His Asp Pro Ser Phe Ile Arg Asn Arg Leu Ser Phe His Phe Phe 85 90 95	

Page 16

Glu Gln Ile Gly Val Leu Ala Pro Ala Ala Ser His Val Phe Leu Tyr 100 105 110

Ile Asn Glu Lys Lys Glu Gly Ile Tyr Leu Lys Ile Glu Ser Val Asp 115 120 125 Asp His Phe Leu Lys Arg Arg Asn Leu Glu Arg Gly Ala Ile Tyr Tyr 130 135 140 Ala Val Asp Asp Asp Ala Asn Phe Ser Leu Leu Ser Ser Phe Asn Lys 145 150 155 Lys Ala Lys Gln Asn Leu Met Gln Gly Tyr Glu Arg Lys Thr Gly Ser 165 170 175 Ser Arg His Asp Asp Tyr Leu His Glu Phe Ile Tyr Phe Ile Asn Thr 180 185 Ala Lys Asp Asp Ile Phe Glu Lys Glu Ile Lys Arg Tyr Leu Asp Val Lys Gln Tyr Leu Leu Trp Leu Ile Gly Ala Val Cys Thr Gln Asn Phe 210 215 220 Asp Gly Phe Val His Asn Tyr Ala Leu Tyr Leu Asn Gly Arg Thr Lys 230 235 240 Thr Phe Gln Ile Ile Pro Trp Asp Tyr Asp Ala Thr Trp Gly Arg Asn 245 250 255 Ile His Gly Glu Glu Met Glu His Asn Arg Ile Pro Ala Lys Gly Tyr 260 265 270 Asn Thr Leu Ser Ala Arg Leu Leu Asp Ile Pro Ala Phe Gln Ser Gln 275 280 285 Tyr Phe Asn Leu Met Lys Asn Val Leu His Arg Gln Phe Thr Ile Ser 290 295 300 Arg Leu Ser Arg Tyr Ala Val Asn Trp His Glu Thr Ile Ala Pro Phe 305 310 315 320 Leu Glu His Asp Pro Tyr Thr Thr Val Thr Tyr Ser Arg Leu Glu Asp 325 330 335 Glu Gln Lys Gln Ile Phe His Phe Ile Asp Gln Arg Lys Arg Phe Leu 340 345 350 Leu Phe Glu Leu Ser Arg 355

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		ļ	Met Trp L	Leu Ty	yr Glu Li 5	ys Lys	s Leu G	10 10	r Pro	
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gtt ac Val Ar caa ta Gln Ty aac ca Asn Gl	gg gtg aga g val Arg 15 ic ggc gga r Gly Gly 30 ig cgc tac	gaa tgo Glu Cys gcg gao Ala Asi	det Trp L aat co Asn Po C ggc ga C GIy G 3! A ccc ga C Pro A	cg aga ro Arg 20 aa ttg lu Leu	ctt gcc Leu Ala gct gcg Ala Ala	aaa t Lys F gcg c Ala L gga c Gly L	tt ttg he Leu 25 tt cgc eu Arg	att Ile tat Tyr	gag Glu ttg Leu	581
gtt ag Val Ar caa ta Gln Ty aac ca Asn Gl	gg gtg aga g val Arg 15 ic ggc gga r Gly Gly 30 ig cgc tac in Arg Tyr	gaa tge Glu Cys gcg gae Ala Asp agc ata	det Trp L L aat Co G Asn Po C ggc gg G Gly G 3! A CCC gg E Pro As	cg aga ro Arg 20 aa ttg lu Leu at aaa sp Lys	ctt gcc Leu Ala gct gcg Ala Ala gtc gtc Val Val	aaa t Lys F gcg c Ala L gga c Gly L	tt ttg he Leu 25 tt cgc leu Arg 10 ttg ctg leu Leu	att Ile tat Tyr acg Thr	gag Glu ttg Leu gac Asp	581 629 677
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Phe Thr Ala Thr Tyr Ile Gln Ala Lys Gly Asp Pro Ile Ala Asp Leu 115 120 125

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Page 20

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Page 22

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٧a٦	٧a٦	Asp 35	Glu	Glу	Lys	Lys	Gly 40	Leu	Phe	Gly	Ile	Phe 45	GТу	His	Arg
ser	Ala 50	۷a٦	٧a٦	Asn	Ile	Arg 55	Glu	Lys	Ile	Asp	Pro 60	٧a٦	Lys	G1u	Ala
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Leu	Glu	Thr 115	Leu	Thr	Gln	Leu	Va1 120	Leu	Asn	Arg	His	Ser 125	Asp	Arg	Tyr
īle	G]n 130	ΑÏα	val	Val	Asp	Ala 135	GTu	Glý	Туг	Arg	140	Lys	Arg	Lys	G] u
Thr 145	Leu	аlа	Gln	Leu	Ala 150	Leu	Lys	Leu	ΑΊa	Asp 155	G]n	ΑΊа	ΑΊа	Arg	Gln 160
Lys	Lys	Asp	Ile	Нis 165	Leu	Glu	Pro	Met	Pro 170	ser	Ser	Glu	Arg	Lys 175	۷a٦

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Tyr Lys Glu Leu Phe Leu Ser Phe Glu Val Leu Glu Ile Leu Ser Val 35 40 45
Leu Phe Trp Phe Ile Gly Val Gly Met Ile Phe Ser Val Ile Ala Gln 50 55 60
Met Gly Phe Val Ile Phe Leu Thr Ile His Arg Phe Ala Leu Glu Ile 65 70 75 80
Phe Arg Ser His Ser Leu Trp Asn Ser Ile Gln Leu Phe Leu Ile Ile 85 90 95
Phe Val Ala Phe Asp Leu Val Tyr Leu Arg Phe Leu Phe Phe Glu Lys 100 105 110
Asp Gly Gly Ser Ile Ile Pro Tyr Ile Trp Leu Pro Leu Phe Ile Leu 115 120 125
Ala val Gly Ile Ala Ala Ala Tyr Ala Lys Gln Lys Gln Ser Ser Lys 130 135 140
130 135 140
Lys Thr Phe Val Ser Ala Leu Phe Leu Met Phe Val Phe Thr Val Met 145 150 155 160
145 150 155 160 Page 33

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Thr Asn Asp Pro Asp Gln Ala Phe Lys Trp Ile Asp Thr Gly Phe Lys 260 270

Lys Ala Gln Glu Val Asn Ser Glu Ile Phe Glu Leu Lys Phe Lys Thr 275 280 285

Leu Tyr Thr Leu His Ser Asp Cys Gln Asn Lys Leu Glu Val Ile Lys 290 295 300

Asp Phe Ile His Gln Leu Glu Asp Lys Lys Ala Trp Val Asp Leu Glu 305 310 315

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<222> (501)..(1598)

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tcgg	jaaga	aa g	gagc	tcca	t ate Me	g aad t Asi	c aa n Ly	g at	c gc e Al 5	c gc	g ga a Gl	a gaa u Glu	a gtg u Va	gc l Al 10	c aac a Asn	533
atc Ile	ctt Leu	Asn	aca Thr 15	tgg Trp	tac Tyr	cgc ( Arg	Ala	atc Ile 20	aga Arg	aga Arg	aat Asn	Wah i	gct ( Ala ( 25	gaa Glu	cag Gln	581
tcg Ser	atc Ile	cga Arg 30	ata Ile	ttt Phe	gaa Glu	Glu	gtc Val 35	aaa Lys	ccg Pro	atg Met	ctg Leu	gca Ala 40	gag : Glu	atg Met	gag Glu	629
gaa Glu	gac Asp 45	caa Gln	gag Glu	gtt Val	tta Leu	atc Ile 50	tac Tyr	tat Tyr	tct Ser	ctg Leu	ctg Leu 55	gaa Glu	ctg Leu	cgg Arg	cat His	677
aaa Lys 60	atc Ile	atg Met	ctg Leu	tat Tyr	gat Asp 65	acg Thr	cgg Arg	gga Gly	aaa Lys	aag Lys 70	ata Ile	gaa Glu	cag Gln	caa Gln	gag Glu 75	725
gag Glu	tta Leu	acg Thr	aac Asn	ggc Gly 80	ggc Gly	agt Ser	gct Ala	gca Ala	tca Ser 85	cat His	atg Met	aca Thr	tcc Ser	tat Tyr 90	tac Tyr	773
tac Tyr	tac Tyr	ctg Leu	ttt Phe 95	tca Ser	gga Gly	gct Ala	tat Tyr	gaa Glu 100	gtg Val	tat Tyr	aaa Lys	aag Lys	aat Asn 105	tat Tyr	gag Glu	821
cag Gln	gcg Ala	atc Ile 110	agc ser	ttc Phe	tat Tyr	aaa Lys	att Ile 115	gcc Ala	gag Glu	aag Lys	aag Lys	ctt Leu 120	gct Ala	cat His	gta Val	869
cat His	gat Asp 125	gaa Glu	att Ile	gag Glu	gtg Val	gcg Ala 130	caa Gln	ttt Phe	cac His	gat Asp	aaa Lys 135	gtc val	gga Gly	aag Lys	ctc Leu	917
tac Tyr 140	· Tyr	tac Tyr	ttg Leu	ggc Gly	cag Gln 145	aat Asn	atc Ile	gtc Val	tct Ser	tta Leu 150	aac Asn	cat His	acc Thr	cgg Arg	cag Gln 155	965
gcg Ala	atg Met	gaa Glu	att Ile	ttc Phe 160	Lys	ggg	cat His	ggc Gly	gac Asp 165	<b>DI3</b>	gat Asp	atg Met	aac Asn	ctt Leu 170		1013
tco Ser	act Thr	tat Tyr	att 11e 175	acg Thr	atg Met	gcc Ala	gga Gly	aat Asn 180	ıyr	aca Thr	gag Glu	atg Met	ggg Gly 185	aaa Lys	tat Tyr	1061
aca Thi	gag Glu	gcg Ala 190	gaa Glu	gaa Glu	tat Tyr	tta Leu	aca Thr 195	Glu	gcc	atc	cat His	acg Thr 200	gta Val	aga Arg	aaa Lys	1109
gce Ala	ggc a Gly 205	' Asp	tgt Cys	ttt Phe	aaa Lys	gaa Glu 210	меτ	cag Gln	ctc Leu	ctt Leu	cat His 215	ASII	ttt Phe	gcc Ala	ttg Leu	1157
ct Lei 220	ı Tyr	gcg Ala	gcg	atg Met	gac Asp 225	AST	tcg Ser	gaa Glu	aaa Lys	agc Ser 230	Tie	cag Gln	ttt Phe	tta Leu	gaa GTu 235	1205
ate	gtt Val	ttg Leu	gat Asp	gat Asp 240	GIn	gca Ala	tat Tyr	gct Ala	gca Ala 245	ı ser	gat Asp	tat Tyr	tat Tyr	Phe 250	aat Asn	1253
gc Ala	t gto a Val	ttt Phe	tta Leu 255	Met	ato : Ile	aaa Lys	gag Glu	cto Lei 260	ı Phe )	e Lys	va	gga Gly	gac Asp 265	MIS	aaa Lys	1301
									Pa	ge 5	U					

	1349
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gca gga gat ggt gaa cag gcg gtt aaa gac tgc aaa gac aac att gaa Ala Gly Asp Gly Glu Gln Ala Val Lys Asp Cys Lys Asp Asn Ile Glu 300 305 310	1445
atc ctg ttt caa aca aag caa tac gac agc gcc aga gaa ctt tcg ctc Ile Leu Phe Gln Thr Lys Gln Tyr Asp Ser Ala Arg Glu Leu Ser Leu 320 325 330	1493
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gag gga atg tgatgaaaaa actgttcatt gttgctgcga ttgctgccgt Glu Gly Met 365	1638
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<211> 366

<212> PRT

<213> Bacillus licheniformis

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Tyr Arg Ala Ile Arg Arg Asn Asp Ala Glu Gln Ser Ile Arg Ile Phe 20 25 30

Glu Glu Val Lys Pro Met Leu Ala Glu Met Glu Glu Asp Gln Glu Val 45

Page 51

Leu Ile Tyr Tyr Ser Leu Leu Glu Leu Arg His Lys Ile Met Leu Tyr 50 60 Asp Thr Arg Gly Lys Lys Ile Glu Gln Gln Glu Glu Leu Thr Asn Gly 65 70 75 80 Gly Ser Ala Ala Ser His Met Thr Ser Tyr Tyr Tyr Leu Phe Ser Gly Ala Tyr Glu Val Tyr Lys Lys Asn Tyr Glu Gln Ala Ile Ser Phe 100 105 110 Tyr Lys Ile Ala Glu Lys Lys Leu Ala His Val His Asp Glu Ile Glu 115 120 125 val Ala Gln Phe His Asp Lys Val Gly Lys Leu Tyr Tyr Tyr Leu Gly 130 140 Gln Asn Ile Val Ser Leu Asn His Thr Arg Gln Ala Met Glu Ile Phe 145 150 155 160 Lys Gly His Gly Asp His Asp Met Asn Leu Val Ser Thr Tyr Ile Thr 165 170 Met Ala Gly Asn Tyr Thr Glu Met Gly Lys Tyr Thr Glu Ala Glu Glu 180 185 190 Tyr Leu Thr Glu Ala Ile His Thr Val Arg Lys Ala Gly Asp Cys Phe
195 200 205 Lys Glu Met Gln Leu Leu His Asn Phe Ala Leu Leu Tyr Ala Ala Met 210 220 Asp Asn Ser Glu Lys Ser Ile Gln Phe Leu Glu Ile Val Leu Asp Asp 225 230 235 Gln Ala Tyr Ala Ala Ser Asp Tyr Tyr Phe Asn Ala Val Phe Leu Met 245 250 255 Ile Lys Glu Leu Phe Lys Val Gly Asp His Lys Arg Ala Ala Ala Phe 260 265 270 Tyr Lys Glu Gly Lys Glu Arg Ser Lys Ser Ala Ala Asn Lys Ile Phe 275 280 285 Asp Ala Lys Ile Asp Ile Leu Tyr Ala Ala Tyr Ala Gly Asp Gly Glu 290 295 300 Gln Ala Val Lys Asp Cys Lys Asp Asn Ile Glu Ile Leu Phe Gln Thr 305 310 315 320 Page 52

Lys	GΊn	Туг	Asp	Ser 325	ΑΊа	Arg	Glu	Leu	Ser 330	Leu	Leu	Thr	Ala	Asn 335	Val	
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<212> DNA

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tccgctatgc gcataaattt gtggagaagc atatttgtta ttctcatctg ttcgttcacg	360
	413
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tot otg gaa aaa ato gog gaa aga tat gaa gtg gao ttt gaa gaa otg	461
Ser Leu Glu Lys Ile Ala Glu Arg Tyr Glu Val Asp Phe Glu Glu Leu 15 20 25	
aaa aag ctg aat tcg cag ctg agc aat cca gac ttg atc atg ccg ggc	509
Lys Lys Leu Asn Ser Gln Leu Ser Asn Pro Asp Leu Ile Met Pro Gly	
atg aaa atc aaa gta ccg tca ggg gga gtg ccg gtc aaa aaa gaa gaa	557
Met Lys île Lys Vaî Pro ser Gîy Gîy Vaî Pro Val Lys Lys Glu Glu 45 50 55	
cag ctc aat atg cga aag gaa tta ccg aaa aaa cag cag gaa cat cca	605
Gin Leu Asn Met Arg Lys Glu Leu Pro Lys Lys Gln Gln Glu His Pro 60 65 70	
ttt gca aaa gaa aag ccg aaa agc aag ctt gat gtt gaa gat ata aaa	653

Page 53

	Phe 75	Ala	Lys	Glu	Lys	Pro 80	Lys	ser	102 Lys	95.9 Leu	ST25 . Asp 85	txt Val	Glu	Asp	Ile	Lys 90	
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	gga Gly	caa Gln	tca Ser	agt Ser 110	ttg Leu	cct Pro	gaa Glu	ggc Gly	gac Asp 115	att Ile	tcg Ser	aat Asn	ttg Leu	tat Tyr 120	caa Gln	agc Ser	749
	gtc Val	aat Asn	cag Gln 125	ctt Leu	cat His	cag G1n	ccg Pro	tac Tyr 130	gta Val	cct Pro	cca Pro	aaa Lys	cct Pro 135	tat Tyr	gaa Glu	cat His	797
	caa Gln	gag Glu 140	aaa Lys	ggc Gly	CCC Pro	aac Asn	atg Met 145	tat Tyr	aat Asn	cca Pro	tgg Trp	aca Thr 150	aat Asn	gag Glu	gag Glu	gaa Glu	845
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	gta Val	gct Ala	gca Ala	gcg Ala 190	Gly	tat Tyr	cat His	cac His	cat His 195	cca Pro	tat Tyr	cct Pro	tat Tyr	ccg Pro 200	Pne	tat Tyr	989
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	ttg Leu	tgc Cys 220	cat His	cct Pro	tgg Trp	tat Tyr	cca Pro 225	tat Tyr	cct Pro	gct Ala	caa Gln	atg Met 230	Pro	tat Tyr	atg Met	cat His	1085
	cag Gln 235	Pro	agc Ser	tat Tyr	gta Val	tct Ser 240	cct Pro	gct Ala	gaa Glu	tat Tyr	gac Asp 245	gat Asp	gat Asp	gac Asp	aac Asn	atg Met 250	1133
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_	gac Asp	tgc Cys 300	<b>99</b> 9	tgc Cys	ggg Gly	ccg Pro	ggc Gly 305	caa Gln	ttc	CCG	gga Gly	ggt Gly 310	ttt Phe	CCa Pro	ggt	gcg Ala	1325
	gcg Ala 315	Pro	tat Tyr	gga Gly	cag Gln	atg Met 320	Pro	caa Gln	atg Met	gga G1y	gct Ala 325	Pro	tac Tyr	ggt Gly	atg Met	999 61y 330	1373
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# Asp Asp Glu Asp 350

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cggggggtac						1653
gcaggtctat						1713
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<212> PRT

<213> Bacillus licheniformis

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Ser Gly Gly Val Pro Val Lys Lys Glu Glu Gln Leu Asn Met Arg Lys 50 60

Glu Leu Pro Lys Lys Gln Gln Glu His Pro Phe Ala Lys Glu Lys Pro 75

Lys Ser Lys Leu Asp Val Glu Asp Ile Lys Pro Lys Glu Lys Pro Ser 90 95

val Pro Tyr Val Pro Pro Val Pro Asn Ile Gly Gln Ser Ser Leu Pro
100 105 110

Glu Gly Asp Ile Ser Asn Leu Tyr Gln Ser Val Asn Gln Leu His Gln 115 120 125

Pro Tyr Val Pro Pro Lys Pro Tyr Glu His Gln Glu Lys Gly Pro Asn 130 135 140 Met Tyr Asn Pro Trp Thr Asn Glu Glu Glu Asn His Met Glu Asn Val 145 150 155 Asn Tyr Pro Asn Val Pro Gln Pro Pro Asn Val Gly Ala Ala Gly Asp 165 170 Glu Asn Lys Gln Phe His Gly Met Pro Asn Val Ala Ala Gly Tyr 180 185 190 His His His Pro Tyr Pro Tyr Pro Phe Tyr Pro Gly Gly Cys Trp Ile 195 200 205 Pro Val Ser Pro Val Leu Pro Gly Ser Gly Leu Cys His Pro Trp Tyr 210 220 Pro Tyr Pro Ala Gln Met Pro Tyr Met His Gln Pro Ser Tyr Val Ser 225 230 235 Pro Ala Glu Tyr Asp Asp Asp Asp Asn Met Gly His Asp Asn Ala Gly 250 255 His His Gly Tyr His His Gln Pro Met Thr Ala Pro Ala Tyr Ala Pro 260 265 270 Tyr Gln Pro Phe Pro Gly Phe Ala Pro Pro Asn Val Gly His Ala Gly 275 280 285 Asp Pro Asn Met Ala His Gly Lys Glu Asp Asp Cys Gly Cys Gly Pro 290 295 300 Gly Gln Phe Pro Gly Gly Phe Pro Gly Ala Ala Pro Tyr Gly Gln Met 305 310 315 Pro Gln Met Gly Ala Pro Tyr Gly Met Gly Gly Tyr Gly Gln Gln Pro 325 335 Ala Gly Gly Gln Met Phe Asn Arg Pro Glu Asp Asp Glu Asp 340 345 <210> 39 <211> 2027

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att gaa ccg Ile Glu Pro	aga gcc c Arg Ala L 15	tg gaa tat eu Glu Tyr	ccg ctt Pro Leu 20	gga aaa g Gly Lys G	ag ctg a Slu Leu a 25	agg gat Arg Asp	581
aaa ttt tca Lys Phe Ser 30	aac atg g Asn Met G	ga ctt gag ily Leu Glu 35	atc agg Ile Arg	GIU Thr I	ict tca Thr Ser 10	cac aad His Asm	629
cag gtg agg Gln Val Arg 45	aat atc c Asn Ile P	cg ggg gaa Pro Gly Glu 50	ggc cac Gly His	ctg caa a Leu Gln L 55	aaa tac .ys Tyr /	aga aat Arg Asr	677
gcg aaa tcc Ala Lys Ser 60	act ttg g Thr Leu V	tg atc ggc al Ile Gly 55	gtc aga Val Arg	aaa aca t Lys Thr l 70	ttg aag eu Lys	ttc gat Phe Asp 75	725
tcg tca aaa Ser Ser Lys	ccg tcc g Pro Ser A 80	gca gaa tac la Glu Tyr	gcg atc Ala Ile 85	ccg ttt g Pro Phe A	ala inr	ggg tgi Gly Cys 90	
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ccg tat atc Pro Tyr Ile 110	aga acg t Arg Thr T	ac gtc aat Tyr Val Asn 115	val Glu	Giu Tie i	ctt gag Leu Glu 120	cag gcg Gln Ala	g 869
gat caa tat Asp Gln Tyr 125	ata aaa g Ile Lys G	gaa agg gct Slu Arg Ala 130	ccc gaa Pro Glu	gat acg of Asp Thr A	cgg ttt Arg Phe	gaa gc Glu Ala	917 a
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ttc gtg aca Phe Val Thr	aaa ttt d Lys Phe H 175	cat cat gtc His His Val	gac cat Asp His 180	ttg ctc (	gat gcc Asp Ala 185	aag ca Lys Hi	c 1061 s
			<b>—</b> — — —				

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cgc Arg 300	Tyr	aaa Lys	tgg Trp	ggc Gly	aga Arg 305	tac Tyr	ggg Gly	att Ile	ggc Gly	aaa Lys 310	tac Tyr	att Ile	tat Tyr	cag Gln	aag Lys 315	1445
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															cagctg	1966
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Ala Leu Pro Gln His Ala Arg Arg Asp Ile Thr Phe Glu Met Ile Gln 260 265

His Arg Phe Thr Lys Pro Ala Lys Arg Val Ile Glu Lys Asn Tyr Pro 275 280 285

Lys Thr Lys Leu Glu Leu Asp Glu Glu Lys Arg Arg Tyr Lys Trp Gly 290 295 300

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aca Thr	ccg Pro	ctc Leu 110	ggt Gly	att Ile	aaa Lys	gcg Ala	atg Met 115	gaa Glu	cag Gln	atg Met	aag Lys	gcg Ala 120	ctc Leu	aac Asn	cgg Arg	869	9
aac Asn	cgc Arg 125	Arg	gaa Glu	gcg Ala	agc Ser	cgc Arg 130	tca Ser	atg Met	cac His	cca Pro	ggc Gly 135					90	5
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629

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Ser Asp Asn His Leu Thr Ile Thr Leu Gln Thr Asp Gly Pro Asp Asp 130 135 140	
Arg Leu Val Ile Phe Leu Asp Phe His Gly Val Phe Thr Lys Leu Thr 145 150 155 160	
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Asp Glu Gly Leu Ser Thr Leu Met Asp Phe Arg Tyr Gln Arg His Phe 50 60

Lys Ala Ala Arg Gly Glu His Gly Met Ser Lys Asn Gln His Gly Arg 65 70 75 80

Asn Ala Glu Asp Met Val Val Lys Val Pro Pro Gly Thr Val Val Ile 85 90 95

Asp Asp Asp Thr Lys Gln Val Ile Ala Asp Leu Thr Glu His Gly Gln 100 105 110

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ctg caa gac ttg cgg ccc aac cct tat cag cca aga aaa acg ttt gat Leu Gln Asp Leu Arg Pro Asn Pro Tyr Gln Pro Arg Lys Thr Phe Asp 30 35 40	629
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ttg cgg ctg ctg aca ctt cct gaa gac gtt caa aag tta atc gac aac Page 66	1013

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Bacillus licheniformis <213>

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120

180 240

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360

420

480

533

581

629

677

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740 745 750 Gly Pro Arg His Val Glu Asn His Asp Leu Trp-Met Lys Arg Lys Leu 755 760 Lys Ser Leu Lys Thr Glu Glu Pro Gln Glu Ile Ala Asp Leu Ile Met 770 780 Glu Glu Val Ile Arg Thr Arg Ser Gly Leu Ile Glu Asp Asp Met Thr 785 790 795 800

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tca agc atc gtt ctg ttt atg ttt aca cct ttt tca ccg tac gtc ctt Ser Ser Ile Val Leu Phe Met Phe Thr Pro Phe Ser Pro Tyr Val Leu 45 50 55	677
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186719271928

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Lys Ar	g Arg 35	Leu	īТе	Leu	GТу	д1а 40	Phe	val	ΑΊa	Ser	Ser 45	Ile	val	Leu
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Leu Se 65	r Phe	ser	٧a٦	va1 70	Ile	val	Leu	val	д1а 75	Phe	GТу	Phe	Lys	Arg 80
Phe Ar	g Phe	Phe	Leu 85	Gln	Asn	Leu	Phe	Ser 90	Phe	туг	Phe	Аlа	т <b>h</b> r 95	Phe
Leu Me	et Gly	Gly 100	Glу	Ile	Ile	Gly	Ala 105	His	ser	Leu	Leu	Glu 110	Thr	Asp
Ser Il	ie Met 115	Glu	Asn	Glу	<b>v</b> a1	Phe 120	Met	Thr	Asn	Trp	ser 125	Gly	Phe	Gly
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Tyr <b>G</b>	lu Glu	Arg	Va1 165	Arg	Leu	Glu	ı Ala	Cys 170	Ile	Gly	Glu	His	Thr 175	Leu
His P	he Thr	Gly 180	Leu	IJе	Asp	Ser	GTy 185	Asn	Gln	Leu	Tyr	Asp 190	Pro	Ile

Thr Lys Thr Pro Val Met Ile Val Asn Ile Glu Lys Leu Lys Val Val 195 200 205 Leu Gly Glu Glu Ala Ser Val Thr Ile Lys Glu Met Ser Pro Leu Asp 210 215 220 Ala Val Gly Lys Leu Asp Glu Ala Leu Pro Tyr Ile Gly Arg Ile Arg 225 230 235 Leu Ile Pro Tyr Arg Gly Val Gly His Gln His Gln Phe Leu Leu Cys 245 250 255 Leu Lys Pro Asp His Val Leu Val Cys Thr Glu Arg Glu Val Ile Glu 260 265 270 Ala Pro Lys Cys Leu Ile Gly Ile Ser Thr Ser Pro Leu Ser Ala Asp 275 280 285 Gly Glu Phe Asp Ala Ile Val His Pro Lys Met Leu Ala Gly Asn Pro 290 295 300 val Lys His Val Ser 305 <210> 52 1922 <211> <212> DNA Bacillus licheniformis <213> <220> <221> **CDS** (501)..(1421) <222> <223> <400> 52

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gaa Glu	ttg Leu	aaa Lys	aaa Lys	gga Gly 80	tat Tyr	gtc val	acg Thr	atc Ile	aga Arg 85	ggc Gly	gga Gly	cac His	cgc Arg	gtg Val 90	ggg Gly	773
ctt Leu	gcc Ala	ggc Gly	cgg Arg 95	gtt Val	gtc Val	gtc Val	gaa Glu	aac Asn 100	ggg Gly	gcc Ala	gtc Val	aaa Lys	gga Gly 105	atc Ile	aga Arg	821
gaa Glu	ata Ile	tca Ser 110	tca Ser	ttt Phe	aat Asn	att Ile	cgc Arg 115	att Ile	gcc Ala	aaa Lys	gaa Glu	aaa Lys 120	atc Ile	ggc Gly	att Ile	869
tcc ser	aaa Lys 125	ccg Pro	tat Tyr	gtc Val	ccc Pro	cat His 130	tta Leu	ttt Phe	caa Gln	aac Asn	tcg Ser 135	tgg Trp	ctg Leu	aac Asn	acg Thr	917
ctg Leu 140	att Ile	atc Ile	ggt Gly	CCG Pro	ccg Pro 145	caa Gln	acc Thr	gga Gly	aaa Lys	aca Thr 150	aca Thr	ctg Leu	ctc Leu	aga Arg	gac Asp 155	∙965
ctc Leu	gcc Ala	agg Arg	ctg Leu	atc Ile 160	agt Ser	tcg Ser	gga Gly	agc Ser	ggc Gly 165	aac Asn	gcc Ala	cct Pro	gcc Ala	aaa Lys 170	aaa Lys	1013
gtg Val	ggg Gly	att Ile	gtt Val 175	gac Asp	gaa Glu	agg Arg	tct Ser	gaa Glu 180	Ile	gca Ala	ggc Gly	tgt Cys	gta Val 185	aac Asn	ggc Gly	1061
ata Ile	ccg Pro	caa Gln 190	Tyr	⊂gg Arg	ctc Leu	ggc Gly	gac Asp 195	Arg	gca Ala	gac Asp	atc Ile	ctt Leu 200	ASP	gcc Ala	tgt Cys	1109
cca Pro	aaa Lys 205	Ala	gaa Glu	999 G1y	ctg Leu	atg Met 210	мет	atg Met	atc Ile	aga Arg	tcg Ser 215	Met	agt Ser	ccg Pro	gag Glu	1157
gta Val 220	atg Met	atc Ile	gcc Ala	gat Asp	gag Glu 225	Ile	ggg Gly	aga Arg	atg Met	gaa Glu 230	: ASP	gca Ala	gaa Glu	gcg Ala	ctc Leu 235	1205
ttg Leu	gaa Glu	gcg	gtc Val	cac His 240	: Ala	ggg	gtg Val	act Thr	gtc Val 245	Tie	gtt Val	tcg Ser	gct Ala	cac His 250	GIY	1253
tac Tyr	aca Thr	tat Tyr	gca Ala 255	gat Asp	ctc Leu	gcc	agg Arg	cgt Arg 260	Pro	tca Ser	' Leu -	aaa Lys	atg Met 265	Leu	caa Gln	1301

gag cac cgg gtt ttt gag cga atc gtg gaa ctt tcc aga aag aac ggt Glu His Arg Val Phe Glu Arg Ile Val Glu Leu Ser Arg Lys Asn Gly 270 275	1349
ccc ggc agc ctg agc cgc atc cta aat ggg aac gga gag ccg ctc ggg Pro Gly Ser Leu Ser Arg Ile Leu Asn Gly Asn Gly Glu Pro Leu Gly 285	1397
gca gca aag agg atg tta tca tgc tgaagctttt aggtgccgtg cttattttgg Ala Ala Lys Arg Met Leu Ser Cys 300 305	1451
cagcagccac atggacagga tttgaaatgg cgaagccttt cagggaaagg ccgaagcaaa	1511
tccgccagct gttggccgct ttgcagtctt tggaggctga aatcatgtac gggcatacac	1571
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tggcgttaac ccatttagag acagaggaag ctgaagcaaa tctcgcccag gcgaaaaatg	1871
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Arg Thr Ser Arg Pro Leu Glu Leu Val Asn Lys Gly Lys Pro Arg Phe 35 40 45	
Leu Pro Tyr Val Ala Thr Pro Glu Asp Ser Ala Leu Leu Leu Asn Arg 50 55 60	
Leu Gly Asn Tyr Ser Met Tyr Thr Leu Glu Glu Glu Leu Lys Lys Gly 65 70 75 80	
Tyr Val Thr Ile Arg Gly Gly His Arg Val Gly Leu Ala Gly Arg Val 85 90 95	
Val Val Glu Asm Gly Ala Val Lys Gly Ile Arg Glu Ile Ser Ser Phe	
100 105 110	

Asn Ile Arg Ile Ala Lys Glu Lys Ile Gly Ile Ser Lys Pro Tyr Val 115 120 125 Pro His Leu Phe Gln Asn Ser Trp Leu Asn Thr Leu Ile Ile Gly Pro 130 140 Pro Gln Thr Gly Lys Thr Thr Leu Leu Arg Asp Leu Ala Arg Leu Ile 145 150 160 Ser Ser Gly Ser Gly Asn Ala Pro Ala Lys Lys Val Gly Ile Val Asp 170 175 Glu Arg Ser Glu Ile Ala Gly Cys Val Asn Gly Ile Pro Gln Tyr Arg 180 185 190 Leu Gly Asp Arg Ala Asp Ile Leu Asp Ala Cys Pro Lys Ala Glu Gly 195 200 205 Leu Met Met Met Ile Arg Ser Met Ser Pro Glu Val Met Ile Ala Asp 210 220 Glu Ile Gly Arg Met Glu Asp Ala Glu Ala Leu Leu Glu Ala Val His 225 230 235 240 Ala Gly Val Thr Val Ile Val Ser Ala His Gly Tyr Thr Tyr Ala Asp 245 250 255 Leu Ala Arg Arg Pro Ser Leu Lys Met Leu Gln Glu His Arg Val Phe 260 265 270 Glu Arg Ile Val Glu Leu Ser Arg Lys Asn Gly Pro Gly Ser Leu Ser 275 280 285 Arg Ile Leu Asn Gly Asn Gly Glu Pro Leu Gly Ala Ala Lys Arg Met 290 295 300 Leu Ser Cys 305

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<211> 1511

<212> DNA

<213> Bacillus licheniformis

<220>

<221> CDS

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	ggtc C													120
	ggaa g													180
	aggct g													240
	ctgtc <b>c</b>													300
	cgccg a													360
	ggtga C													420
	attga a													480
	cggtc <b>c</b>													530
gcaaa	gagga <b>t</b>	gttat	мет	ctg a Leu L	ys L	eu L	eu G	ly A	la v	al L		le i	.eu	
			1					000	224	cct	_		gaa	578
gca g Ala A	ca gcc la Ala	inri	rp Thr	Gly	Phe	Glu 20	Met	ĂĨā	Lys	Pro	Phe 25	Ārģ	ĞÎü	
		15			<b>.</b>		200	act	++0	cad	-	tta	gag	626
agg C Arg P	cg aag ro Lys	Gln 3	cle Arg	Gln	Leu	Leu	Ala	Αla	Leu	Gln 40	Ser	Leu	์ ดีโน	
	30				35	c.c.a	ctc	cat	cad	-	tca	ааа	cao	674
Ala G	aa atc lu Ile	Met T	ryr Gly	<u> </u>	Thr	Pro	Leu	Arg-	Gln 55	Āla	Ser	Lys	GTn	
	.5			50		a+-	acc	+++		+++	cau	aca	ttt	722
atc g Ile A	ca cac la His	Gln	Leu <u>In</u> r	Glu	Pro	val	Ala	Ser 70	Leu	Phe	GÌn	Thr	Phe 75	
60			65				+	_	<b>550</b>	266	uca	taa		770
gca g Ala G	aa cag lu Gln	Leu	Gin ras	Gly	agc Ser	Ala	žer	Ala	Gly	Thr	Ala	Trp 90	ĞĨü	,,,
		İ	80				00					50		818
gac a	igc ctg	gag	aaa gta	ı rgg	כנכ	yaa	Pag	ge 83	3	uuu	uug		<i>5</i> -	'

Asp	Ser	Leu	Glu 95	Lys '	val -	Trp I	Pro	102 Glu 100	95.s Thr	т25. Ala	txt Leu	Lys	Lys 105	Lys	Glu	
tac Tyr	gag Glu	att Ile 110	tta Leu	cgg Arg	caa Gln	rne '	ggc Gly 115	gaa Glu	acg Thr	ctg Leu	ggc Gly	cgt Arg 120	cat His	gat Asp	ctg Leu	866
att Ile	tct Ser 125		caa Gln	aaa Lys	cat His	atc Ile 130	aaa Lys	ctg Leu	gcg Ala	tta Leu	acc Thr 135	cat His	tta Leu	gag Glu	aca Thr	914
gag Glu 140	gaa Glu	gct Ala	gaa Glu	gca Ala	aat Asn 145	ctc Leu	gcc Ala	cag Gln	gcg Ala	aaa Lys 150	aat Asn	gaa Glu	aaa Lys	atg Met	gtc Val 155	962
	agc ser	ctt Leu	gga Gly	ttt Phe 160	ttg Leu	acg Thr	gga Gly	ctg Leu	cta Leu 165	ctg Leu	att Ile	ctt Leu	cta Leu	ttg Leu 170	atg Met	1010
taa	tgaa	gag	ggga		ac ac	gaa	atg Met	gga Gly	gta Val	gac Asp 175	gta Val	aat Asn	att Ile	att Ile	ttt Phe 180	1062
caa Gln	att Ile	gcc Ala	ggc Gly	gtc Val 185	ggg Gly	atc Ile	gtc Val	gtc Val	gct Ala 190	ttt Phe	ctt Leu	cac His	acc Thr	ata Ile 195		1110
gat Asp	caa Gln	atg Met	ggg Gly 200	Lys	aag Lys	gaa Glu	tat Tyr	gcc Ala 205	caa Gln	tgg Trp	gtc Val	acg Thr	ctt Leu 210		gga Gly	1158
ttc Phe	att lle	tat Tyr 215	. Tie	ttg Leu	ttc Phe	atg Met	gtg Val 220	AIG	act	gtt Val	gtc Val	gat Asp 225		cta Leu	ttc Phe	1206
caa Glr	a aag n Lys 230	ata Ile	222	gct Ala	gtc Val	ttt Phe 235	cta Leu	ttt Phe	caa Gln	gga	tag /	19999	gct	cact	c att Ile 240	1257
gaa Glu	a ato		caa Glr	a ato 11e 245	vai	gga Gly	ctg	g gga u Gly	atg Met 250		gco Ala	aco The	tto Phe	c cto e Leu 25!		1305
tte Lei		gtg Va	aaa Lys 260	5 GIL	caa Gln	aaa Lys	ccg	acg Thr 265	PHE	gc1	t tti a Phe	tte e Lei	ati 110 270		gtt Val	1353
tt: Ph	t gco e Ala	gg a Gly 27	y Cys	acg Thi	att Ile	ttt Phe	tta Lei 280	J PNE	tta Lei	gt: J Va	a gat 1 Ası	cae Gli 28		c ta l Ty	c gaa r Glu	1401
at Il	c att e Ilo 290	e Ar	g ate	g att	t gaa e Glu	aaa Lys 295	) T1	a gci e Ala	t gcc a Ala	a as	t gc n Ala 30	× 710	c at n Il	c aa e As	c atg n Met	1449
at Me 30	g ta		c ga 1 G1	a acq u Thi	g ati r Ile 310	s rer	jaa Ly	g ati	t ate	c gg e G1 31	y	t gc e Al	t ta a Ty	t at r Il	t gcg e-Ala 320	1497
ga	g tt u Ph	t gg e Gl	c gc y Al	c ca a												1511

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<212> PRT

<213> Bacillus licheniformis

<400> 55

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Arg Gln Leu Leu Ala Ala Leu Gln Ser Leu Glu Ala Glu Ile Met Tyr 35 40 45

Gly His Thr Pro Leu Arg Gln Ala Ser Lys Gln Ile Ala His Gln Leu 50 60

Thr Glu Pro Val Ala Ser Leu Phe Gln Thr Phe Ala Glu Gln Leu Glu 65 70 75 80

Lys Gly Ser Ala Ser Ala Gly Thr Ala Trp Glu Asp Ser Leu Glu Lys 90 95

Val Trp Pro Glu Thr Ala Leu Lys Lys Glu Tyr Glu Ile Leu Arg 100 105 110

Gln Phe Gly Glu Thr Leu Gly Arg His Asp Leu Ile Ser Gln Gln Lys 115 120 125

His Ile Lys Leu Ala Leu Thr His Leu Glu Thr Glu Glu Ala Glu Ala 130 135 140

Asn Leu Ala Gln Ala Lys Asn Glu Lys Met Val Lys Ser Leu Gly Phe 145 150 160

Leu Thr Gly Leu Leu Leu Ile Leu Leu Met 165

<210> 56

<211> 68

<212> PRT

<213> Bacillus licheniformis

<400> 56

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20 25 30

Tyr Ala Gln Trp Val Thr Leu Leu Gly Phe Ile Tyr Ile Leu Phe Met 35 40

Val Ala Thr Val Val Asp Asp Leu Phe Gln Lys Ile Lys Ala Val Phe 50 60

Leu Phe Gln Gly

<210> 57

<211> 85

<212> PRT

<213> Bacillus licheniformis

<400> 57

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Ser Leu Ile Val Lys Glu Gln Lys Pro Thr Phe Ala Phe Leu Ile Val 20 25 30

Val Phe Ala Gly Cys Thr Ile Phe Leu Phe Leu Val Asp Gln Val Tyr 35 40 45

Glu Ile Ile Arg Met Ile Glu Lys Ile Ala Ala Asn Ala Asn Ile Asn 50 60

Met Met Tyr Val Glu Thr Ile Leu Lys Ile Ile Gly Ile Ala Tyr Ile 65 70 75

Ala Glu Phe Gly Ala 85

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ccagctgttg go						120
ccgtcaggca to						180
gacatttgca ga						240
cctggagaaa gi						300
attcggcgaa a						360
gttaacccat t						420
aatggtcaaa a						480
aagagggag c		5+6 663 6t	a nac ota a		tt caa att	533
gcc ggc gtc Ala Gly Val	ggg atc gt Gly Ile Va 15	c gtc gct	ttt ctt cac Phe Leu His 20	acc ata ctg Thr Ile Leu 25	gat caa Asp Gln	581
atg ggg aag Met Gly Lys 30	aag gaa ta Lys Glu Ty	at gcc caa /r Ala Gln 35	tgg gtc acg Trp Val Thr	ctt tta gga Leu Leu Gly 40	ttc att Phe Ile	629
tat ata ttq		tg gca act	att atc gat	gat cta tto Asp Leu Pho 55	caa aag	677
ata aaa gct Ile Lys Ala 60	gtc ttt c val Phe L 6	eu Phe Gin	gga taggggg Gly	gct cactcati	tga	724
aatcgttcaa a	atcgtaggac	tgggaat <b>g</b> at	t cgccacctto	ctcagcttga	ttgtgaaaga	784
gcaaaaaccg a	acgtttgctt	ttttgattg	t cgtttttgc	ggctgcacga	ttttttatt	844
				a aaaatagctg		904
				c gggattgctt		964
gtttggcgcc (	cagctgacaa	aggatgccg	g acagggtgc	g attgcttcga	agatcgaatt	1024
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				g aggatttcct		1144
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ttc						1207

<210> 59

<211> 68

<212> PRT

<213> Bacillus licheniformis

<400> 59

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Met Gly	val	Asp	va7 5	Asn	Ile	Ile	Phe	Gln 10	Ile	Ala	Gly	Vai	15	116	
val val	Ala	Phe 20	Leu	нis	Thr	Ile	Leu 25	Asp	Gln	Met	Gly	Lys 30	Lys	Glu	
Tyr Ala	a Gln 35	Тгр	val	Thr	Leu	Leu 40	Gly	Phe	IJе	Туг	11e 45	Leu	Phe	Met	
val Al: 50	a Thr	٧a٦	٧a٦	Asp	Asp 55	Leu	Phe	Gln	Lys	11e 60	Lys	Αla	val	Phe	
Leu Ph 65	e Gln	GТу	•												
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ttcaa	ggata	999	gggc	-tca	ctta	anga	.aa :	2222	ccaa	c at	ttac	tttt	tta	attatc	g 180
ccacc	ttcct	cag	cttg	att	gtya	aaya	.gc		natr		itcta	спаа	ato	attgtc attcoo	a 240
														attcgg atttt	
														attttg accaga	
														gccgga	
														atggct	-9
														gtcttag	
cagaa	.aggag	g gat	tttc	ctga	gtg Val 1	aag Lys	cgt Arg	ttt Phe	ctg Leu 5	Phe	Trp	Leu	Leu	gtc at Val II 10	le
gga a Gly l	itc gt :le Va	ta to	ys Pi	tt g he G	ga gg ly A	cg ca la H	at a is A 2	311 Y	ta ca al G	aa g ln A	ct to la S	cg co er P 2		aa gaa ys Glu	581

gcg Ala	gag Glu	ccg Pro 30	gct Ala	ggg Gly	gaa Glu	acc Thr	gct Ala 35	gca Ala	gaa Glu	gaa Glu	tcg Ser	gca Ala 40	gaa Glu	gcc Ala	att Ile	629
gca Ala	aga Arg 45	gag Glu	cag Gln	gct Ala	gaa Glu	ggt Gly 50	ttg Leu	gaa Glu	cta Leu	gac Asp	cgg Arg 55	gtc Val	ggg Gly	gag Glu	ttc Phe	677
tgg Trp 60	aac Asn	aac Asn	att Ile	ttg Leu	aca Thr 65	gag Glu	tat Tyr	ggg Gly	gga Gly	cac His 70	ctt Leu	CCC Pro	gaa Glu	agt Ser	caa Gln 75	725
aaa Lys	gga Gly	agc Ser	ctg Leu	ctt Leu 80	gaa Glu	ttt Phe	gtc val	aaa Lys	gga G1y 85	gaa Glu	aag Lys	cac His	ttt Phe	tcg ser 90	cct Pro	773
gag Glu	gaa Glu	tgg Trp	ggc Gly 95	aaa Lys	gcg Ala	ctg Leu	ttt Phe	tcc ser 100	tac Tyr	ttg Leu	ttc Phe	cat His	gaa Glu 105	gtg Val	ctg Leu	821
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gtc Val	ctg Leu 125	Leu	cag Gln	ctt Leu	ttg Leu	caa Gln 130	aac Asn	gcg Ala	ttt Phe	caa Gln	caa Gln 135	agc Ser	acc Thr	gtc Val	agc Ser	917
aaa Lys 140	: vaı	gcg Ala	tat Tyr	gca Ala	att Ile 145	vai	tac Tyr	atg Met	gtg Val	ctg Leu 150		att Ile	ctt Leu	gcg	ctc Leu 155	965
aac Asr	ago Ser	ttt Phe	cgg Arg	gtt Val 160	Ala	gtc Val	aca Thr	tat Tyr	gcg Ala 165	ASII	gaa Glu	gcg Ala	att Ile	cag Glr 170	acg Thr	1013
ato Me1	aca Thr	ago Ser	ttt Phe	: Ile	ctg Leu	tcg Ser	cto Leu	gta Val 180	PIO	ctg Leu	ctt Leu	cto Lei	g gcg i Ala 185		atg Met	1061
gcg	g act a Thr	tcg Ser 190	ggg Gly	g gga ⁄ Gly	gco Ala	gco Ala	tca Ser 195	gcc Ala	gca Ala	tto Phe	ttt Phe	cat His 200	ccg Pro	gto Val	att   Ile	1109
ct: Le:	t ttt u Phe 205	e Lei	atg Met	aad Asr	acg Thr	ago Ser 210	. G1)	ttg Lei	ttt Phe	ato Ile	caa Glr 21!	יעי י	t ato	gtç Va	ttg Leu	1157
CC Pri 22	o Lei	t tta ı Lei	a tti J Pho	t tta e Leu	tca Ser 225	Ala	ati i Ile	t tta e Leu	a ago u Sei	att 110 230	= va	age I Se	c acg r Thi	ate r Me	acg t Thr 235	1205
ga As	c caa p Gli	a tai	t aaar r Ly:	a gtg s Va 240	<u>I</u> Thi	a cag	g cte	g gco u Ala	c cag a Gli 24!	i rei	c cte	c aga u Ar	a aa¹ g Ası	t gco n Al- 25	g gcg a Ala O	1253
at Il	c gg e Gl	c ac	g ctg r Lei 25	LI Ali	t gca a Ala	a tti a Pho	t ttg e Lei	g aco u The 260	<u> </u>	a tte l Phe	c ct	c gg u Gl	t gto y Va 26	<u></u> .	c tcg e Ser	1301
gt Va	t ca 1 Gl	g gg n G1 27	y Ali	c tca a Se	a gc r Ala	c gc a Ala	a gt a va 27	<u>i in</u>	g ga r As	c gg p G1	c at y Il	t ac e Th 28	i re	g cg u Ar	g acg g Thr	1349
gc AT	a aa a Ly 28	5 Ph	c at e Il	t ac e Th	c gg r Gl	a aa y As 29	n Pn	c ate	е РГ	c gt o Va	29	<u>u</u>	c cg y Ar	c at g Me	g ttt t Phe	1397

acc Thr 300	gaa Glu	gcg Ala	aca Thr	gac Asp	acg Thr 305	gtg Val	atc Ile	agc Ser	gcg Ala	tct Ser 310	ctc Leu	ctg Leu	ctg Leu	aaa Lys	aac Asn 315	1445
acc Thr	gtc Val	ggg Gly	ata Ile	ctc Leu 320	ggt Gly	gtg Val	gca Ala	atc Ile	tta Leu 325	att Ile	tgc Cys	atc Ile	gca Ala	gcc Ala 330	ttt Phe	1493
ccc Pro	gcg Ala	atc Ile	aaa Lys 335	atc Ile	ctt Leu	tcc Ser	ctc Leu	gcg Ala 340	ctc Leu	ata Ile	tac Tyr	aaa Lys	att Ile 345	gcc Ala	gcg Ala	1541
gcg Ala	gtt Val	ctc Leu 350	cag Gln	cct Pro	ctc Leu	gga Gly	ggc Gly 355	ggc Gly	ccg Pro	gtt Val	atc Ile	agc ser 360	tgc Cys	ctg Leu	gat Asp	1589
gtc Val	atc Ile 365	Ser	aaa Lys	agc Ser	gtc Val	atc Ile 370	ıyr	att Ile	ttc Phe	gcg Ala	gcc Ala 375	atg Met	gcc Ala	atc Ile	gtt Val	1637
tcg Ser 380	ctg Leu	ato	ttt Phe	ttc Phe	tta Leu 385	ser	tta Leu	acc Thr	gtg val	atc Ile 390	TIE	aca Thr	gcg Ala	ggg Gly	aat Asn 395	1685
ctg Leu	acg Thr	atg Met	atg Met	atg Met 400	aag Lys	tag	ggag	gga	tgag	atgg	aa t	ttct	gaca	g		1733
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<210> 61

<211> 401

<212> PRT

<213> Bacillus licheniformis

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PAUL 34	

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tat Tyr	cac His	tac Tyr 30	ttt Phe	ctg Leu	ctg Leu	ctt Leu	ttt Phe 35	gtt Val	ctc Leu	ggc Gly	gtt Val	tcc Ser 40	ttc Phe	atg Met	ctc Leu	629
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caa aaa caa gga aaa aaa cgg atc cat ctt aaa ttt gaa ttg tac gga Gln Lys Gln Gly Lys Lys Arg Ile His Leu Lys Phe Glu Leu Tyr Gly 15 20 25	581
tta atc tgt atc gcc atc tcg att att gcg gtt ttg cag ctt ggc gta Leu Ile Cys Ile Ala Ile Ser Ile Ile Ala Val Leu Gln Leu Gly Val 30 35 40	629
gca ggg caa acg ttc att tac atg ttc cgc ttt ttc gcc ggt gaa tgg Ala Gly Gln Thr Phe Ile Tyr Met Phe Arg Phe Phe Ala Gly Glu Trp 45 50 55	677
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tac Tyr	tgc Cys	atc Ile	att Ile 95	gca Ala	agc ser	atg Met	ctg Leu	ctt Leu 100	ctt Leu	tca Ser	cat His	gtc Val	cag Gln 105	ctg Leu	ttt Phe	821
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tca Se	a gaa r Glu	a ccg ı Pro	g att	e Ile	t tca e Ser	a ago Sei	tt1 Phe	t tco ser 260	. Azt	cg¹ Arg	t gat g Ası	t ga p G1	a aag u Ly 26		c gaa o Glu	1301
gt: Va	t ca:	a gc1 n Ala 276	а ту	c gaa r Gli	a gct u Ala	t cc	g gcg 5 A1 27	3 MIC	t cct a Pro	gc Ala	t gaa a Gl	a cc u Pr 28	•	t go o Al	t gag a Glu	1349
CC Pr	c ga o Gli -28	u III	c gg	t gaq y Gli	g gaa u Gl	a ato u Me 29	r an	g gce n Ala	c tce a Se	c gg	c gc y Al 29		c ga o Gl	a at u I	c acg le Thr	1397
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ct Le	g ga u As	t ga p As	t cc p Pr	g aa o Ly 32	S HI	c ac s Th	a gg r Gl	g ca y Gl	g ca n G1 32	<u>.</u> ~.	g ga a As	t aa p Ly	a aa 's Ly		at att sn Ile 30	1493
ta Ty	c ga r As	c aa p As	t gc n Al 33	<u>a</u> ar	g aa g Ly	g ct s Le	g ga u Gl	a ag u Ar 34	8 '''	i Pii		a ag n Se	c tt er Ph 34		ga gtt ly Val	1541
									P	age '	98					

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gad gug gag aga gug gut tru gag gug aga gug gug ata gug gag aga gug gag gag gag gag gag ga	aag Lys	gcg Ala	aaa Lys 350	gtc Val	acc Thr	cag Gln	gtt val	cat His 355	ctc Leu	ggc Gly	ccg Pro	gcc Ala	gtc Val 360	acg Thr	aaa Lys	tat Tyr	1589
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gaa gtg gtg atg gtt teer tee Lew Lys gdu val Lew Glu val Lew Ass 425  gac cag gat gca aag ctg atg ate gtg atg ggc ctc agg aca att tcc atg gg gaa gtg gtg ata gtg gtg aca gac gaa atg ctg aac aag aaa agg gtc att gat gtg ggg aca agg ctc atg gcc gaa gag atg gtg lew Val Ass Glu val Lew Val Ass Glu Ass Ass Ass Ass Ass Ass Ass Ass Ass As	gcc Ala	ccg Pro	atc Ile	ccc Pro	gga Gly 400	aaa Lys	tcg Ser	gcg Ala	att Ile	gga Gly 405	atc Ile	gaa Glu	gtg Val	ccg Pro	aat Asn 410	gcg Ala	1733
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gga gga gga gga gga gga gga gga ggg gga aa gg gg	gac Asp	cgg Arg	Pro	gat Asp	gca Ala	aag Lys	ctg Leu	мет	atc Ile	ggc Gly	ctc Leu	ggc Gly	AI 9	aac Asn	att Ile	tcc Ser	1829
gca ggg ggg acc ggg agg cgg agg cgg cgg	gga Gly	Glu	gcg Ala	gta Val	ttg Leu	gca Ala	GIU	ctg Leu	aac Asn	aaa Lys	atg Met	FIU	nis	ctt Leu	ctt Leu	gtt Val	1877
act agc aft ttg aft agg gld add Lys Pro His glu Val Lys Met Met 480  att gat ccg aaa atg gtc gag ctc aat 1le Asp Pro Lys Met Val Glu Leu Asn 500  ttg ctc gct ccc gtc gtg aca agg gac ccg aaa aaa gca tcg cag gct ttg Leu Ala Pro Val Val Thr Asp Pro Lys Lys Ala Ser Gln Ala Leu 510  aag aaa gtc gtc aac gaa atg gag cgg cgc tac gaa ttg ttt tct cac Lys Lys Val Val Asn Glu Met Glu Arg Tyr Glu Leu Phe Ser His 525  acg gga acg aga aat atc gaa ggg tat Asn Ala Ala Glu Glu Ala Lys Gln Pro 565  aat gcc gca gaa gaa gca aag cag cag cgg cgg	Ala	Gly	gcg Ala	acc Thr	gga Gly	ser	ggg Gly	aaa Lys	agc Ser	gtc Val	CĀZ	Vai	aac Asn	ggg Gly	atc Ile	115	1925
The Asp Pro Lys Met Val Glu Leu Ash Val Tyr Ash Gly Tile Pro His Sobs Leu Leu Ala Pro Val Val Thr Asp Pro Lys Lys Ala Ser Gln Ala Leu Sio	aca Thr	agc Ser	att	ttg Leu	Met	Arg	gca Ala	aag Lys	ccc	H15	GIU	gtg Val	l aag Lys	atg Met	MEL	MEC	1973
Leu Leu Ala Pro Val Val Thr Asp Pro Lys Lys Ala Ser Gin Ala Leu Silo Pro Val Val Thr Asp Pro Silo Pro Lys Lys Ala Ser Gin Ala Leu Silo Pro	att Ile	gat Asp	ccg Pro	Ly5	мет	gtc Val	gag Glu	cto Leu	LAST	ı vaı	tac Tyr	aac Asn	ggg Gly	TIC	FIG	cat His	2021
aag aaa gtc gtc aac gaa gal aar stc gaa ggg tat aac gac tat att aaa cgg atg het ser his sac gga acg aga aat atc gaa ggg tat aac gac tat att aaa cgg atg het ser his sac gac ggg acg ggg tat aac gac tat att aaa cgg atg het ser his sac gac ggg acg gag ctt cca tac atc atc gtg ser	ttg Leu	cto Leu	Ala	Pro	gtc Val	gtg Val	aca Thr	ASP	) Pro	aaa Lys	aaa Lys	gca Ala	ı Ser	GII	gct Ala	ttg Leu	2069
Thr Gly Thr Arg Asn Ile Glu Gly Tyr Asn Asp Tyr Ile Lys Arg Met 555  aat gcc gca gaa gaa gca aag cag ccg gag ctt cca tac atc att gtg 2213 Asn Ala Ala Glu Glu Ala Lys Gln Pro Glu Leu Pro Tyr Ile Ile Val 565  att gtg gac gag ctt gcc gac ctg atg atg gtg gct tcc tct gat gtt Ile Val Asp Glu Leu Ala Asp Leu Met Val Ala Ser Ser Asp Val 585  gaa gac tcg atc aca agg ctt tcg caa atg gcc agg gcg gcg gcg gcg gcg gcg gcg gc		Lys	: Va I	gtc val	aac Asn	gaa Glu	мет	GIL	cgc Arc	g cgc g Arg	tac Tyr	GIL	3 FEG	ttt Phe	tct Ser	cac His	2117
Asn Ala Ala Glu Glu Ala Lys Gln Pro Glu Leu Pro Tyr Ile Ile Val 560  att gtg gac gag ctt gcc gac ctg atg atg gtc gct tcc tct gat gtt Ile Val Asp Glu Leu Ala Asp Leu Met Val Ala Ser Ser Asp Val 580  gaa gac tcg atc aca agg ctt tcg caa atg gcc agg gcg gcg gcg gcg atc Ser Ser Ile Thr Arg Leu Ser Gln Met Ala Arg Ala Ala Gly Ile 595  cac ctg atc att gcg acg cag agg cct tcg gtc gat gtt atc aca ggg cac ctg atc aca agg cct tcg gtc gat gtt atc aca ggg 2357  His Leu Ile Ile Ala Thr Gln Arg Pro Ser Val Asp Val Ile Thr Gly 615	Thr	GIY	acg Thr	aga Arg	aat J Asr	Tie	GIU	ggg	tat Tyr	aac Asr	ı VəF	יעוי	t att	aaa Lys	cgg Arg	,	2165
The value of the v	aat Asr	gco	gca	gaa Glu	I GIL	1 VIS	aag Lys	cag Gli	g cc <u>c</u> n Pro	J GIL	1 rer	CCa Pro	a tac o Tyr	ato Ile			2213
Glu Asp Ser Ile Thr Arg Leu Ser Gln Met Ala Arg Ala Ala Gly Ile 590  Cac ctg atc att gcg acg cag agg cct tcg gtc gat gtt atc aca ggg His Leu Ile Ile Ala Thr Gln Arg Pro Ser Val Asp Val Ile Thr Gly 605  615	att Ile	gto Val	gad Asp	) GIL	i Fer	gco L Ala	gad Asp	cte	J Met	c met	g gto	gc	t tco a Sei	261	_ ^5	gtt val	2261
His Leu Ile Ile Ala Thr Gln Arg Pro Ser Val Asp Val Ile Thr Gly 605 610 615	gaa Glu	a gad u Asp	Ser	· 116	aca Thi	a agg r Arg	cti Lei	ı Se	r GII	a atg n Mei	g gco t Ala	ag a Ar	y Ale	AIG	g gge a Gly	atc / Ile	2309
Page 99	cae Hi	s Lei	i Ile	ati	t gce a Ala	g aco a Thi	· GII	<u>ı Ar</u>	g cci g Pro	o Sei	r va	61	p va	t ato	c aca	a ggg r Gly	2357

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cag Glr	acc Thr	gac Asp	tcc Ser	agg Arg 640	acg Thr	att Ile	ctt Leu	gat Asp	atg Met 645	gga Gly	ggc Gly	gct Ala	gaa Glu	aaa Lys 650	ctt Leu	2453
cto	ggc Gly	aga Arg	ggg G1y 655	gac Asp	atg Met	ctg Leu	ttt Phe	ctc Leu 660	cct Pro	gtc Val	ggc Gly	gcc Ala	aat Asn 665	aaa Lys	CCG Pro	2501
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gt: Va	gat Asp 685	cac His	gtc Val	atc Ile	agc Ser	cag Gln 690	caa Gln	aaa Lys	gcc Ala	caa Gln	tac Tyr 695	caa Gln	gaa Glu	gaa Glu	atg Met	2597
at 11 70	cca Pro	gaa Glu	gag Glu	acg Thr	cag Gln 705	gaa Glu	acg Thr	gtc Val	agc Ser	gaa Glu 710	gtg Val	aca Thr	gac Asp	gac Asp	ctt Leu 715	2645
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ct Le	t atc u Ile	gat Asp 750	gcc Ala	atg Met	gaa Glu	gag Glu	cgg Arg 755	gga Gly	atc Ile	gtc Val	ggc Gly	cca Pro 760	tat Tyr	gaa Glu	gga Gly	2789
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gc	tcccg	agt	gaat	ttga	igc t	gtca	aaga	t go	ttgg	tgtg	ago	:agaa	ıcgg	cttt	gcgtga	3133
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Ser Ser Phe Ser Asp Arg Asp Glu Lys Pro Glu Val Gln Ala Tyr Glu 260 265 270 Ala Pro Ala Ala Pro Ala Glu Pro Pro Ala Glu Pro Glu Ile Gly Glu 275 280 285 Glu Met Gln Ala Ser Gly Ala Pro Glu Ile Thr Phe Thr Glu Leu Glu 290 295 300 Asn Lys Asp Tyr Gln Leu Pro Ser Ile Gln Leu Leu Asp Asp Pro Lys 305 310 315 His Thr Gly Gln Gln Ala Asp Lys Lys Asn Ile Tyr Asp Asn Ala Arg 325 330 335 Lys Leu Glu Arg Thr Phe Gln Ser Phe Gly Val Lys Ala Lys Val Thr Gln Val His Leu Gly Pro Ala Val Thr Lys Tyr Glu Val Tyr Pro Asp 355 360 365 Val Gly Val Lys Val Ser Lys Ile Val Asn Leu Ser Asp Asp Leu Ala 370 380 Leu Ala Leu Ala Ala Lys Asp Ile Arg Ile Glu Ala Pro Ile Pro Gly 385 390 395 400 Lys Ser Ala Ile Gly Ile Glu Val Pro Asn Ala Glu Val Ala Met Val 405 410 415 Ser Leu Lys Glu Val Leu Glu Ser Lys Leu Asn Asp Arg Pro Asp Ala 420 425 430 Lys Leu Met Ile Gly Leu Gly Arg Asn Ile Ser Gly Glu Ala Val Leu 435 445 Ala Glu Leu Asn Lys Met Pro His Leu Leu Val Ala Gly Ala Thr Gly
450 460 Ser Gly Lys Ser Val Cys Val Asn Gly Ile Ile Thr Ser Ile Leu Met 465 470 475 480 Arg Ala Lys Pro His Glu Val Lys Met Met Met Ile Asp Pro Lys Met 485 490 495 Val Glu Leu Asn Val Tyr Asn Gly Ile Pro His Leu Leu Ala Pro Val 500 505 510 Val Thr Asp Pro Lys Lys Ala Ser Gln Ala Leu Lys Lys Val Val Asn 515 520 525 Page 102

Glu Met Glu Arg Arg Tyr Glu Leu Phe Ser His Thr Gly Thr Arg Asn 530 540 Ile Glu Gly Tyr Asn Asp Tyr Ile Lys Arg Met Asn Ala Ala Glu Glu 545 550 560 Ala Lys Gln Pro Glu Leu Pro Tyr Ile Ile Val Ile Val Asp Glu Leu 565 570 Ala Asp Leu Met Met Val Ala Ser Ser Asp Val Glu Asp Ser Ile Thr 580 585 590 Arg Leu Ser Gln Met Ala Arg Ala Ala Gly Ile His Leu Ile Ile Ala 595 600 605 Thr Gln Arg Pro Ser Val Asp Val Ile Thr Gly Val Ile Lys Ala Asn 610 620 Ile Pro Ser Arg Ile Ala Phe Ser Val Ser Ser Gln Thr Asp Ser Arg 625 630 640 Thr Ile Leu Asp Met Gly Gly Ala Glu Lys Leu Leu Gly Arg Gly Asp 645 650 Met Leu Phe Leu Pro Val Gly Ala Asn Lys Pro Leu Arg Val Gln Gly 660 670 Ala Phe Leu Ser Asp Glu Glu Val Glu Lys Val Val Asp His Val Ile 675 680 685 Ser Gln Gln Lys Ala Gln Tyr Gln Glu Glu Met Ile Pro Glu Glu Thr 690 695 700 Gln Glu Thr Val Ser Glu Val Thr Asp Asp Leu Tyr Asp Glu Ala Val 705 710 720 Ala Leu Val Val Ser Met Gln Thr Ala Ser Val Ser Met Leu Gln Arg 725 730 735 Arg Phe Arg Ile Gly Tyr Thr Arg Ala Ala Arg Leu Ile Asp Ala Met
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Met Arg Lys Pro Thr Ile Lys Glu Leu Ile Phe
1 5 10 533 caa cat atg aag gac cat ctg tcg atc tat tta ttt gtt tct gtg ctg Gln His Met Lys Asp His Leu Ser Ile Tyr Leu Phe Val Ser Val Leu 15 20 25 581 ttc tta atg ggt gtg att ttc ggc gcg gtc atc gtc aac agc atg acg Phe Leu Met Gly Val Ile Phe Gly Ala Val Ile Val Asn Ser Met Thr 30 35 40 629 atc ggt caa aaa gaa gat ttg ttc tac tat ttg aat caa ttt ttt gga Ile Gly Gln Lys Glu Asp Leu Phe Tyr Tyr Leu Asn Gln Phe Phe Gly 45 50 55 677 cag ctt tcc gaa gga aaa gca gcc agc tca aag gaa atg ttt ttg cag Gln Leu Ser Glu Gly Lys Ala Ala Ser Ser Lys Glu Met Phe Leu Gln 60 65 70 75 725 agc ttt ctt cat aat atg aaa tat tta ggc tta atg tgg att ctc ggg Ser Phe Leu His Asn Met Lys-Tyr Leu Gly Leu Met Trp Ile Leu Gly 80 85 773 ata tcc atc atc ggt ctg ccc gtc att ttt atc atg gtc ttc tta aaa Ile Ser Ile Ile Gly Leu Pro Val Ile Phe Ile Met Val Phe Leu Lys 95 100 821 ggg atc gtc gga ttt aca gtc ggc ttt ttg gtc aat caa atg gga Gly Ile Val Val Gly Phe Thr Val Gly Phe Leu Val Asn Gln Met Gly 110 115 120 869 917 atc aac ggc ttt ttc ctg tct ttt gtc tcc gtg ctc ccg caa aat att

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Ile	Asn 125	Gly	Phe	Phe	Leu	Ser 130	Phe	102 val	95.9 Ser	т25. Val	txt Leu 135	Pro	<b>G</b> In	Asn	Ile	
ctg Leu 140	ctg Leu	atc Ile	ccg Pro	gcg Ala	tac Tyr 145	ttg Leu	atc Ile	atg Met	ggc Gly	acc Thr 150	tgc Cys	gcc Ala	atc Ile	gcc Ala	ttt Phe 155	965
tcg Ser	atg Met	agg Arg	ctc Leu	atc Ile 160	cgc Arg	cag Gln	ctt Leu	ttt Phe	gta Val 165	aac Asn	gca Ala	gcc Ala	ttc Phe	aga Arg 170	agc Ser	1013
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หวัร	Leu	Ser	11e 20	туг	Leu	Phe	val	ser 25	۷a٦	Leu	Phe	Leu	Met 30	GТу	val	
IJе	Phe	e G]y 35	Ala	٧a٦	Ile	val	Asn 40	Ser	Met	Thr	Ile	G]y 45	Gln	Lys	Glu	
Asp	Leu 50	Phe	Tyr	туг	Leu	Asn 55	Gln	Phe	Phe	σΊу	G]n 60	Leu	ser	Glu	Glу	
Lys 65	Ala	Ala	Ser	Ser	Lys 70	Glu	Met	Phe	Leu	G]n 75	Ser	Phe	Leu	His	Asn 80	
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Leu	Pro	val	17e 100		Ile	Met	Val	Phe 105		Lys	Gly	Ile	Va7 110	Va]	GТу	
 Phe	"Thr	• Val 115		Phe	Leu	va-1	Asn 120		Met	Gly	: Ile	. Asn 125	_G7.y	_Phe	Ph <u>e</u>	·· -··
Leu	Ser   130		val	Ser	va1	Leu 135		Gln	Asn	Ile	Leu 140		Ile	Pro	Ala	

Tyr Leu Ile Met Gly Thr Cys Ala Ile Ala Phe Ser Met Arg Leu Ile 145 150 155 160

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val Pro Arg Val Ala 180

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<211> 1108

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cgt cag cct gct gct cgt ttt tat ttt atc agg cgt gct cac atc gct Arg Gln Pro Ala Ala Arg Phe Tyr Phe Ile Arg Arg Ala His Ile Ala 30 35 40	628
gcg tcc tca gtt aag gcc gtc ttc atc gtt gta ccg ggt ggc tca tca Ala ser Ser Val Lys Ala Val Phe Ile Val Val Pro Gly Gly Ser Ser 45 50 55	676
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ctt tgc atc aga att gcc gga acc gaa tca gcg ctt tca gct ttc ccc Leu Cys Ile Arg Ile Ala Gly Thr Glu Ser Ala Leu Ser Ala Phe Pro 80 85 90 Page 106	772
- ·	

cct	cgt Arg	cct	gaa Glu	gct Ala	ggc Gly	gac Asp	cag Gln	cat His	tca Ser	ttt Phe	gaa Glu	aga Arg	tcc Ser	gcg Ala	aag Lys	820
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Arg Asp Gly Leu Tyr Glu Tyr Ala Val Arg Ile Ser Ala Ala Asp Ser 130 135	
Gly Leu Lys Arg Gly Glu Arg Ser Glu Ser Cys Arg Ala Ser Arg Glu 145 150 160	
Thr Glu Lys Glu Asn Arg Arg Gly Lys Thr Ser Ser Thr Ile Asp Gly 165	
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Page 108	

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190 Ala Gly Lys Asn Leu Tyr Asn Glu Glu Gly Gly Asn His Val His Phe 200 205

Glu Ile Arg Lys Asp Gly Val Ala Leu Asn Pro Leu Asn Phe Met Asp 210 215 220

Lys Pro Val Ser Ser Ile Glu Lys Ala Met Glu Glu Gln Ala Ser Glu 225 230 235 240 Page 110

								10.		3123					
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ctt tat gtc atc ggc gcc gtg ata tat tgg acg cac gat ccg cag tca Page 111	677

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Ala Val Ile Tyr Trp Thr His Asp Pro Gln Ser Ile Phe Thr Asn Pro 50 60

Leu His Tyr Leu Ile Val Ala Val Phe Phe Thr Leu Thr Asp Ala Phe 65 70 75 80

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Thr Asp Thr Arg Met Leu Leu Glu Glu Asn Asn Asp Leu Leu His Thr 100 105 110

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Ile His Ile Tyr Tyr Gly Asn Ile Glu Ala Tyr Ala Glu Gly Ile Glu 130 135 140

Lys Leu Ile Lys Arg Phe Ala Glu Lys Met Asn Ile Ser Ala Ala Leu 145 150 155 160

Cys Glu Tyr Asn Ser Glu Glu Ser Lys Asp His Leu Leu Glu His Met 165 170 175

Glu Asn Arg Phe Asp Val Gln Glu Lys Leu Asp Arg Lys Asp Val Tyr 180 185 190

Tyr Glu Glu Asn Gly Lys Met Val Leu Ile Pro Phe Ser Ile His Asp 195 200 205

Phe Asp Tyr Val Met Lys Leu Thr Ser Glu Asp Leu Val Thr Glu Phe 210 220 Page 113

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Cgc cct gca atg caa aaa ctt att tca gat gtt gaa aag gat aaa ttt Arg Pro Ala Met Gln Lys Leu Ile Ser Asp Val Glu Lys Asp Lys Phe 65 70 75	725
caa gct gtt ctt gtt tgg aag atc tca cgc cta tca cga aat atg tta Page 114	773

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Asp Glu Gly Il	e Ser Gly Lys Asn 55	Ile Ser Gly Ar 60	g Pro Ala Met Gin	
Lys Leu Ile Se 65	r Asp Val Glu Lys 70	Asp Lys Phe Gl 75	n Ala Val Leu Val 80	· · · ·
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275 280 285 Phe Leu Leu Ser Ser Leu Leu Arg Cys Pro Asp Cys Gly Gln Gly Met 290 295 300 Val Pro Ala Ile Thr Thr Asn Lys Arg Lys Asp Gly Thr Lys Lys 305 310 315 Tyr Arg Tyr Tyr Val Cys Ser Asn Phe His Asn Lys Gly Ser Ser Ala 325 330 335 Asn Lys Ile Glu Lys Ile Leu Ser Asn Gln Asn Gln Leu Phe Ser Lys 355 360 365 Leu Gln Ser Ile Asn Thr Thr Ser Ile Glu Ser Leu Asn Gln Leu Asn 370 380

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ccc ttt tca tca gac Pro Phe Ser Ser Asp 25	ggc aaa aag cgg ( Gly Lys Lys Arg ( 30	ccg ctc aag ccg ( Pro Leu Lys Pro l 35	cct gca tgg 629 Pro Ala Trp 40	
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gta cct gcc gta gga aag gtt cag gag aaa ttc tca ggg cag ggc att Val Pro Ala Val Gly Lys Val Gln Glu Lys Phe Ser Gly Gln Gly Ile 155 160 165	1013
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<400> 79

<213> Bacillus licheniformis

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	ca ccg cct tat er Pro Pro Tyr 20	gtc ctg aac tgt atc Val Leu Asn Cys Ile 25	1059
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	tc ctg atc tcc le Leu Ile Ser	gct ctg tta aca ggc Ala Leu Leu Thr Gly 70	1203
ctt ggc gtc tac gac aga atc g	ga cag ttt gcc Page 12:		1251

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Val Phe His Phe Asp Glu Lys Thr Ala Gly Asn Pro Thr Val Ala Thr 50 55 60	
Leu Ile Leu Ile Ser Ala Leu Leu Thr Gly Leu Gly Val Tyr Asp Arg 65 70 75 80	
Ile Gly Gln Phe Ala Gly Ala Gly Ser Ala Val Pro Val Thr Gly Phe	* <b>-</b> -
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Val Leu Gly Val Trp Thr Asn Met Phe Lys Leu Ala Gly Asn Val Ile 115 120 125	
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aaa gtt aaa gcg tat cag cct tca ccg cct tat gtc ctg aac tgt atc Lys Val Lys Ala Tyr Gln Pro Ser Pro Pro Tyr Val Leu Asn Cys Ile 20	
15	629
aag gca ttt ctc gtc ggt ggt ctg att tgt acg atc ggc cag gct ttc Lys Ala Phe Leu Val Gly Gly Leu Ile Cys Thr Ile Gly Gln Ala Phe 35	
30 acc qcc qqq	677
cag aat ttt tat atg gct gtg ttc cat ttt gat gaa aaa acg gcc ggg Gln Asn Phe Tyr Met Ala Val Phe His Phe Asp Glu Lys Thr Ala Gly 50	
4)	725
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ctt gcg gga aac gtc atc gtg ttc ggt gtt gtg gcc gct tat att gtg Leu Ala Gly Asn Val Ile Val Phe Gly Val Val Ala Ala Tyr Ile Va 125 130 135	g 917 I
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ttc	1453
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Val Phe His Phe Asp Glu Lys Thr Ala Gly Asn Pro Thr Val Ala Thr 50 60

Leu Ile Leu Ile Ser Ala Leu Leu Thr Gly Leu Gly Val Tyr Asp Arg 65 70 75 80

Ile Gly Gln Phe Ala Gly Ala Gly Ser Ala Val Pro Val Thr Gly Phe 85 90 95 Page 124

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Val Leu Gly Val Trp Thr Asn Met Phe Lys Leu Ala Gly Asn Val Ile 115 120 125	
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tcgtgttcgg tgttgtggcc gcttatattg tggggatgat ccgctttgcc tttgacaagc 480	,
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gaa aaa gaa ggt cct ctt gga cac tta ttt gac aaa agc tat gat gaa 629 Glu Lys Glu Gly Pro Leu Gly His Leu Phe Asp Lys Ser Tyr Asp Glu 30 35 40	,
atg cac tgc aac cag aaa aac tgg gaa atg gca gag cgc aag ctg atg 677 Page 125	,

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gac Asp	atc Ile	gat Asp	atc Ile	ttt Phe 80	ttg Leu	gct Ala	ggc Gly	gat Asp	ctg Leu 85	ctc Leu	aac Asn	caa Gln	aac Asn	gtg Val 90	aca Thr		773
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																	1397
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130 aat gat Asp Gly Gly Phe Ala Lys 130 aat gat Asp Cyg gga Aca Gag Cag Ash Ala Thr Ala Glu Arg Gln Lys Pro Gly Thr Ala Thr Gy Cag Asp Cag	gag gat gcg gtt cag ser Ala Leu gcg act ttg gag gat gcg ggg ggg ggg ggg ggg ggg ggg ggg act acg	Met His Cys Asn Gln Lys Asn Trp Glu gag gat gcg gtt cag tcc gcg tta tca gac atc gat atc ttt ttg gct ggc gat Asp Tle Asp Tle Phe Leu Ala Gly Asp gca atc tat gtg gcg cgg tta ttg aaa Ala Asn Tyr val Ala Arg His Leu lys gga gca tgc tcg aca tca atg gat gcg loo gad gca tgc tcg aca tca atg gaa tcg lle Asp Gly Gly Phe Ala lys Arg Ala 115 Ser  att gac ggg ggt ttc gaa aaa cgc gcc gcc lle Asp Gly Gly Phe Ala lys Arg Ala 125 Gly Phe Ala lys Arg Ala 125 Gly Phe Ala lys Arg Ala 125 Gly Phe Ala 125 Gly Phe Ala 126 Gly Arg 127 Gly Phe Ala 130 Fly 128 Gly Phe Ala 130 Fly 130 F	Met His Cys Asn Gln Lys Asn Trp Glu Met Glu Asp Ala Val Gln Ser Ala Leu Ser Lys Glu Asp Ala Val Gln Ser Ala Leu Ser Lys Glu Asp Ala Val Gln Ser Ala Leu Ser Lys Glu Asp Ile Phe Leu Ala Gly Asp Leu Ser Casp Caac Ala Leu Ser Lys Bo Ser Ala Leu Ser Lys Ala Ala Asn Tyr Val Ala Arg His Leu Lys Ile Lou Lys Ile Lys Ile Asp Gly Gly Phe Ala Lys Arg Ala Leu Lys Arg Ala Leu Asp Gly Gly Phe Ala Lys Arg Ala Leu Lys Arg Ala Leu Lys Asp Gly Gly Phe Ala Lys Arg Ala Leu Lys Arg Ala Leu Lys Arg Ala Leu Lys Arg Ala Leu Lys Arg Ala Casp Cag Cag Cag Cag Cag Cag Gln Lys Pro Gly Thro Ala Thr Ser Thr Val Lys Arg Ala Leu Lys Arg Ala Leu Ser Gln Lys Pro Gly Thro Ala Thr Ser Thr Val Lys Lys Cag Arg Cag Lyg Gly Fro Gly Fro Gly Fro Gly Gly Fro Asp Leu Gly Arg Val Leu Ser Cag Cag Cag Cag Ggg att acc gat Gly Arg The Pro Asp Asp Thr Ile Cag Gly Gly Arg Thr Pro Asp Leu Cag Gly Gly Arg Thr Lys Asp Thr Ile Cag Gly Gly Cag Cag Cag Cag Cag Cag Cag Gly Gly Fro Asp Cys Cag	Met         His         Cys         Asn         Gln         Lys         Asn         Trp         Glu         Met         Ala           gag         gat         gct         gtt         caa         gce         tta         tca         aaa         caa           Glu         Asp         Ala         val         Gln         65s         Ala         Leu         ser         Lys         Gln           gag         atc         gat         gtc         gtc         tta         tca         aaa         atc         gln         gtc         ctc         ctc         aaa         att         gln         pro         gln         ctc         gaa         att         gcc         ctc         gaa         att         gcc         gcc         gaa         att         gcc         gcc	45         50         55           gag gat gat gat gat gat gat gat gat gat	Met         His         Cys         Asn         Gln         Lys         Asn         Trp         Glu         Met         Ala         Glu         Arg           gag         gat         gog         gtt         cag         tcc         gcg         tta         tca         aaa         caa         aat         ctt         td         gfl         Asp         rfl         Asp         rfl         chr         ttt         gfl         ggc         ggc         gat         tca         caa         caa	Met His Cys Asn Gin Lys Asn Trp Glu Met Ala Glu Arg Lys Glu Asp Ala Val Gin Ser Ala Leu Ser Lys Glin Asn Ceu Lys Glu Asp Ala Val Gin Ser Ala Leu Ser Lys Glin Asn Ceu Lys Glu Asp Ala Val Gin Ser Ala Leu Ser Lys Glin Asn Ceu Lys Glu Asp Ala Val Gin Ser Ala Leu Ser Lys Glin Asn Ceu Lys Glu Asp Ala Val Gin Asn Ceu Lys Glin Asn Ceu Lys Glin Asn Ceu Lys Glin Asn Ceu Lys Glin Asn Ceu Lys Asp Tie Asp Tie Phe Leu Ala Gly Asp Leu Leu Asn Glin Cys Ser Thr Ser Met Glin Ser Tie Ala Glin Cys Ser Tie Ala Tie Ser Ser Ile Ala Tie Ser Ser Ile Asn Glin Asn Glin Asn Glin Cys Asp Glin Cys Asp Glin Cys Asp Glin Cys Fro Glin Glin Cys Fro Glin Asn Glin Cys Fro Glin Asn Glin Cys Fro Glin Glin Cys Glin Glin Cys Fro Glin Glin Cys Fro Glin Glin Cys Fro Glin Glin	MET         His Cys         Asn         Gl         Lys         Asn         Trp         Glu         Met         Ala         Glu         Asp         Ala         Glu         Leu         Leu         Ser         Leu         Leu         Ser         Leu         Leu<	MET         His Cys         Asn Gln         Lys         Asn Trp         Glu         Met         Ala         Glu         Arg         Lys         Leu         Met         Ala         Glu         Arg         Lys         Glu         Arg         Leu         Arg         Gra         Arg         Gra         Arg         Gra         Arg         Gra         Leu         Arg         Ctc         Caa         Caa         Caa         Cut         Lys         Glu         Met         Alg         Ctc         Caa         Caa         Cut         Lys         Arg         Leu         Arg         Ctc         Ctc         Ctc         Ctc         Arg         Arg         Leu         Arg         Arg         Leu         Arg         Arg         Arg	Met His Cys Asn Gln Lys Asn Trp Glu Met Ala Glu Arg Lys Leu Met 45 glu gcg gtt cag scc gcg tta tca aaa gca aat ctt aaa aag gaa gcg gat cag scc gcg tta tca aaa aag caa aat ctt aaa aag gaa acg atc gat atc gat 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35 40 45	
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Lys Asn Trp Glu Met Ala Glu Arg Lys Leu Met Glu Asp Ala Val Gln 50 55 60	
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Lys Asn Trp Glu Met Ala Glu Arg Lys Leu Met Glu Asp Ala Val Gln 50  Ser Ala Leu Ser Lys Gln Asn Leu Lys Lys Glu Asp Ile Asp Ile Phe 65  Leu Ala Gly Asp Leu Leu Asn Gln Asn Val Thr Ala Asn Tyr Val Ala	
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Lys Asn Trp Glu Met Ala Glu Arg Lys Leu Met Glu Asp Ala Val Gln 550 Ser Ala Leu Ser Lys Gln Asn Leu Lys Lys Glu Asp Ile Asp Ile Phe 65 70 Asp Leu Asn Gln Asn Val Thr Ala Asn Tyr Val Ala Gly Asp Leu Lys Ile Pro Phe Leu Cys Leu Phe Gly Ala Cys Ser Thr	

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aaa Lys	gaa Glu	19 at 11	•	aa ( lu /	gat Asp	gtg Val	aag Lys 210		gac Asp	: g;	gg (	ctg Leu	CCG Pro 215	_	g t	cg	ga1 AS	t a	aa ys	1157
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g A	la Me				at i Il	c gc e Al	a <u>~</u>	t g la V	ta t al L	tg .eu	ate	c gg e G1		at sp 75	ato Ile	gc Al	g a	itc []e	aat Asn	1637
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Tyr Tyr Val Asn Gly Leu Cys Asp Thr Gln Tyr Ile Ile His Leu Leu 50 60

Arg Glu Leu Val His Leu Asn Asp Lys Glu Lys Glu Ser Gly Glu Val 65 70 75 80

Glu Asp Ile Val Glu Asn Arg Leu Leu Asn Gln Gln Val Ser Lys Ala 85 90 95 Glu Thr Leu Asp Glu Ala Val Asp Gln Val Leu Ser Gly Leu Val Ala 100 105 110 Ile Ile Val Glu Asp Ala Gly Phe Ala Phe Ile Ile Asp Val Arg Ser 115 120 125 Tyr Pro Gly Arg Thr Pro Glu Glu Pro Asp Thr Glu Lys Val Val Arg 130 135 140 Gly Ala Arg Asp Gly Leu Val Glu Asn Ile Ile Val Asn Thr Ala Leu 145 150 150 160 Ile Arg Arg Arg Ile Arg Asp Glu Arg Leu Arg Tyr Lys Met Leu His 165 170 175 Ile Gly Glu Arg Ser Lys Thr Asp Ile Cys Leu Cys Tyr Leu Glu Asp 180 185 Val Ala Asp Pro Asp Leu Val Glu Val Leu Lys Lys Glu Ile Glu Asp 195 200 205 Val Lys Ile Asp Gly Leu Pro Met Ser Asp Lys Ser Val Glu Glu Phe 210 215 220 Leu Val Gly Gln Gly Tyr Asn Pro Phe Pro Leu Val Arg Phe Thr Glu 225 230 235 240 Arg Ala Asp Val Ala Ala Ser His Ile Leu Glu Gly His Val Ile Val 245 250 255 Ile Val Asp Thr Ser Pro Ser Val Ile Ile Thr Pro Thr Thr Leu Phe 260 270 His His Val Gln His Ala Glu Glu Tyr Arg Gln Thr Pro Ala Val Gly
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Met Asn Leu Ser Gly Glu Cys Leu Arg Pro Leu Leu Asp Tyr Tyr Glu 65 70 75 80

Ile Pro Val Asp Asn Leu Lys Val Ile Tyr Asp Asp Leu Asp Leu Pro 85 90 95

Thr Gly Arg Ile Arg Leu Arg Thr Lys Gly Ser Ala Gly Gly His Asn 100 105 110

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ggc Gly	aca Thr	tgc Cys	atc Ile 175	att Ile	atg Met	atc Ile	ttt Phe	gtc Val 180	gcg Ala	999 61y	gcg Ala	aga Arg	att Ile 185	tcg Ser	cac His	1061
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Glu Arg Asn Gly Ser Arg Ser Trp Ile Gly Val Gly Ala Phe Ser Ile 100 105 110

Gln Pro Ser Glu Phe Met Lys Leu Ala Met Ile Ala Phe Leu Ala Lys 115 120 125

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Bacillus licheniformis <213>

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ser Pro Val Leu Met Ala Ala Lys Ala Thr Met Arg Asn Ser Arg Pro 110	869
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Page 144

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gc <sup>*</sup> A1: 22:	t aaa		g tt o Ph	t tt e Ph	t aca e The 225	r Ala	tct Ser	tto Lei	tco Sei	g gcg r A1a 230	a Ale	a gaq a Gli	g cgi	t ac	g aag r Lys 235	1205
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Glu Glu Glu Pro Glu Leu Ser His Ser Ser Tyr Gln Pro His Glu Glu 290 295 300 Leu Lys Glu Asn Pro Phe Tyr Ser Val Pro Pro Leu Leu Lys Glu Asp 305 310 315 Gln Asn Asp Arg Glu Pro Glu Ala Phe Glu Val Glu Val Thr Gln Glu 325 330 335 Ala Glu Ala Ile Asp Glu Glu Glu Ala Gly His Thr Ile Glu Ile 340 350 Pro Glu Tyr Ser Phe His Glu Gln Thr Glu Pro Glu Glu Glu Arg Asp 355 360 365 Glu Met Gln Ala Ala Asp Glu Gln Glu Val Ser Ala Lys Glu Asn Asp 370 380 Asn Ala Leu Tyr Leu Thr Lys Leu Phe Thr Lys Gln Gly Glu Glu 385 390 395 Phe Thr Arg Met Arg Met Cys Ile Val Gln Gln Asn Asp Thr Ile Asp 415 Leu Leu Cys Glu Arg Tyr Asp Ile Asn Val Gln Gln Leu Ile Arg Met 420 430 Asn Ser Leu Ser Leu Asp Glu Glu Leu Lys Glu Gly Gln Ile Leu Tyr 435 440 445 Ile Pro Asp Tyr Gln Asn Ser His Ala 450 <210> 98

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aga Arg	aga Arg	aga Arg	gto Val 255	Ala	ago Ser	cgc	tac Tyr	aga Arg 260	יוט ו	991 61 <sub>3</sub>	t ttg / Lei	g aaa u Lys	acg Thr 265		g gag a Glu	1301
cac His	cto Leu	aca Thr 270	. rer	ccg Pro	gca Ala	gag Glu	aaa Lys 275	, GIU	gaq i Asp	Cg(	c aca	a cat r His 280		tai Tyi	cat His	1349
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									Pa	ge 1	しつ4					

Page 154

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Pro Val His Ile Phe Gly Gln Pro Ala Asp Met Asp Glu Ile Met Glu 130 135 140 Page 155	

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aag ctg ata gag cgc aac agc gtc acc gta tat gac aat ttt tca aga Lys Leu Ile Glu Arg Asn Ser Val Thr Val Tyr Asp Asn Phe Ser Arg 30 35 40	629
gac tcc ctc cgg tat aag cct tac cgg gac cat cct cac ttg aaa gtg Asp Ser Leu Arg Tyr Lys Pro Tyr Arg Asp His Pro His Leu Lys Val 45 50 55	677
ctg cag gga gac att ttg gat ttg aac gcg ctt aaa aag gcg atc cag Leu Gln Gly Asp Ile Leu Asp Leu Asn Ala Leu Lys Lys Ala Ile Gln 60 65 70 75	725
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Lys Pro Tyr Arg Asp His Pro His Leu Lys Val Leu Gln Gly Asp Ile 50 60

Leu Asp Leu Asn Ala Leu Lys Lys Ala Ile Gln Gly Ala Ser His Ile 65 70 75 80

val His Ala Ala Gly Ile Ala Gly Ile Asp Thr Val Ile Gln Asn Pro 85 90 95

Val Lys Thr Met Gln Val Asn Met Ile Gly Ser Ala Asn Leu Leu Glu 100 105 110

Ala Ala Gly Leu Thr Glu Cys Lys Arg Val Val Cys Phe Ser Thr 115 120 125

Ser Glu Val Phe Gly Gln Ile Ala Phe Arg Ala Arg Glu Thr Ser His 130 135 140

Thr Val Leu Gly Ala Val Gly Glu Ala Arg Trp Thr Tyr Ala Val Ser 145 150 160

Lys Leu Ala Glu Glu His Met Ala Tyr Ala Tyr Phe Lys Glu Leu Gly 165 170

Leu Pro Thr Val Thr Val Arg Pro Phe Asn Val Tyr Gly Pro Glu Gln 180 185 190

Val Gly Glu Gly Ala Ile Lys Thr Met Val His Arg Ala Leu Leu Asp 195 200 205

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Tyr Val Asp Asp Met Ile Asp Gly Ile Leu Arg Cys Leu Thr Met Lys 235 240 Page 159

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Ser	Gln	11e 275	Phe	Phe	Gly	Glu	Lys 280	Lys	Glu	Ala	Asp	11e 285	Glu	Leu	Arg
Ile	Pro 290	Gln	Val	Asn	Lys	Ala 295	Lys	Glu	Met	Leu	Gly 300	Phe	Ser	Ala	Lys
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<211> 70

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<213> Bacillus licheniformis

<400> 105

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Page 162

1145

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211> 65	
212> PRT	
213> Bacillus licheniformis	
13> Buck Fus Frenchisonalis	
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221> CDS	
222> (501)(1463)	
223>	
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	40
and the second of the second o	00
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raye 103	

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<213> Bacillus licheniformis

<400> 109

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Page 165

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<211> 419

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<213> Bacillus licheniformis

<400> 111

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<212> DNA

Bacillus licheniformis <213>

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(558)..(1511) <222>

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cag ctg tca ttc gaa ctg gag cgg att tcg gca aac aag gaa gac aag Page 171	782

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His Arg Leu His Leu Thr Asn Val Lys Ala Glu Glu Lys Lys Ser 115 120 125

Leu His Ser Gln Ile Glu Tyr Glu Lys Leu His Ala Glu Arg Glu Lys 130 135 140

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Gln Ile Glu Thr Asp Val Ala Val Leu Lys Glu Arg Val Thr Glu Thr 180 185 190

Lys Ser Arg Leu Leu Glu Ala Glu Lys Thr Lys Glu Ala Leu Phe Tyr 195 200 205

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Page 174

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Phe Leu Val Val Val Ile Ala Ser Leu Glu Leu Pro Val Ser Ile Ser 50 60

Lys Phe Ile Ala Glu Ser Asn Pro Lys Leu His Glu Ser Met Leu Lys 70 75 80

His Ala Leu Arg Met Thr Ala Val Cys Thr Val Phe Ser Thr Ala Ala 85 90 95

Ala Val Ile Ile Leu Pro Phe Ile Pro Val Phe Asp Ser Tyr His Pro 100 105 110

Leu Ile Arg Gly Leu Val Ile Gly Met Ile Pro Thr Val Ala Phe Thr 115 120 125

Ser Ile Ala Arg Gly Tyr Phe Met Gly Val Gln Gln Met Gly Lys Ile 130 140

Ala Thr Ala Asn Ala Leu Lys Lys Ile Phe Gln Leu Ile Gly Leu Phe 145 150 155 160

Leu Phe Phe Gln Trp Tyr Ser Phe Glu Leu Asp Thr Ser Leu Leu Ile 165 170 175

Ser Leu Phe Val Leu Val Ala Ser Glu Val Val Val Phe Val Tyr Leu 180 185 190

Phe Ser Gln Phe Val Leu Val Arg Arg Ala Ala Gln Lys Gly Gln Gln 195 200 205 Page 177

Ile His Leu Arg Arg Asn Asp Val Leu Lys Arg Leu Leu Thr Val Ser 210 220 Ile Pro Thr Thr Gly Leu Arg Val Phe His Ala Val Thr Asn Ala Val 225 230 235 240 Glu Pro Phe Leu Val Lys Gly Thr Leu Leu Ala Gly Val Ser Arg 245 250 255 Thr Ser Ala Ile Asp Gln Phe Gly Met Leu Ser Gly Val Ala Met Thr 260 265 270 Ile Gly Phe Phe Pro Ala Phe Ile Ala His Ser Leu Met Val Val Met 275 280 285 Ile Pro Ser Ile Ser Glu Ser Tyr Ala Tyr Gly Gln Tyr Glu Arg Val 290 295 300 Ile Lys Arg Ile Lys Gln Ala Ile Phe Ile Thr Leu Phe Tyr Gly Ile 305 310 315 320 Pro Ser Val Met Val Met Tyr His Phe Ala Glu Pro Leu Thr His Leu 325 330 335 Phe Phe Asp Ser Val Lys Ala Ser Phe Tyr Leu Lys Met Leu Trp Pro 340 345 Tyr Phe Leu Phe His Phe Phe Ala Met Pro Phe Gln Ala Cys Leu Ile 355 360 365 Gly Met Gly Leu Ala Lys Asp Ala Phe Tyr His Asn Val Trp Ala Ser 370 380 Val Leu Ser Phe Leu Met Met Tyr Val Leu Gly Ser Met Gln Thr Leu 385 390 395 400 Gln Met Thr Gly Ile Ile Leu Ala Met Asn Thr Gly Met Ile Leu Leu 405 415 Thr Ala Leu His Tyr Val Thr Ile Cys Lys Glu Leu Gly Val Thr Leu 420 425 430 Phe Leu Thr Asn Lys Ser Arg Ser Pro Arg Ile Glu Ser Arg 445

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50 55 60
Ile Leu Thr Val Tyr Ala Leu Lys His Val Ser Ile Glu Asn Arg Gly
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Gly Val Leu Tyr Phe Arg Thr His Leu Trp Val Glu Leu Ile Val Leu
85 90 95
Phe Leu Phe Leu Tyr Arg Phe Leu Tyr Arg Ile Ala Glu Ile Gly Gln
100 105 110
Leu Gln Thr Ala Val Ser Asp Gly Gly Ser Ala Ala Tyr Gly Ala Leu 115 120 125
Phe Ala Gln Asp Pro Ala Thr Met Ile Gly Phe Phe Val Leu Ala Val 130 135 140
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Met Ile Arg Gly Ile Leu Ile Ala Leu Leu Gly
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acg ctt gcg atg aac agc aaa aaa acg ctg tct ccc gct ttg gcg gaa Thr Leu Ala Met Asn Ser Lys Lys Thr Leu Ser Pro Ala Leu Ala Glu 60 75

gtc tgg aaa acg act tct gaa gcg cat aac aat gtc agc cag ctg ccg

Page 181

725

773

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			att Ile													1397
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Asp Gly Glu Ile Thr Gly Phe Asn Ala Lys Asp Phe Leu Ile Ser His 355 360 365

Lys Lys Arg Asp Leu Pro Lys Pro Lys Leu Thr Pro Glu Lys Ala Lys 370 380

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Phe Asp Cys Lys Cys Arg Phe Ile Lys Arg Lys Gly Phe Pro Ph 65 70 75	he Leu 80
Val Gln Lys Ser Lys Arg Asn Ser Gly Phe Thr Phe Gly Val A 90 95	la Ala S
Phe Phe Ile Ile Met Phe Leu Leu Ser Asn Met Leu Trp Lys I 100 105 110	le Asp
Ile Thr Gly Ala Asn Pro Glu Thr Glu His Gln Ile Arg Gln G 115 120 125 Page 187	ln Leu

Asp Gln Ile Gly Val Lys Lys Gly Arg Phe Gln Phe Ser Met Leu Thr 130 140 Pro Glu Lys Ile Gln Gln Ala Leu Thr Lys Arg Val Glu Asn Ile Thr 145 150 155 160 Trp Val Gly Ile Glu Leu Asn Gly Thr Ala Leu His Met Lys Val Val 175 Glu Lys Asn Glu Pro Asp Lys Glu Lys Tyr Ile Gly Pro Arg His Ile 180 185 190 Val Ala Lys Lys Gly Ala Thr Ile Ser Lys Met Phe Val Glu Lys Gly 195 200 205 Glu Pro Leu Val Thr Val Asn Gln His Val Glu Lys Gly Gln Met Leu 210 220 Val Ser Gly Leu Ile Gly Ser Glu Glu Glu Lys Gln Lys Val Gly Ala 225 230 235 240 Lys Gly Lys Ile Tyr Gly Glu Thr Trp Tyr Lys Ser Thr Val Thr Val 245 250 255 Pro Leu Glu Thr Ser Phe Asp Val Phe Thr Gly Lys Val Arg Thr Ser 260 265 270 His Lys Leu Ser Leu Gly Ser Leu Thr Met Pro Ile Trp Gly Phe Ser 275 280 285 Phe Lys Lys Glu Asp Phe Ser Arg Pro Lys Thr Glu Thr Glu Lys His 290 295 300 Ser Leu His Phe Ile Asn Phe Lys Leu Pro Val Ala Tyr Glu Lys Glu 305 310 315 His Met Arg Glu Ser Glu Gln Ile Lys Arg Val Tyr Ser Lys Lys Glu 325 330 335 Ala Val Leu Arg Arg Asn Arg Asn Gly Lys Lys Arg His Gln Asp Lys 340 345 Asn Arg Gln Arg Glu His Tyr Gln Cys Lys Ser Phe Ala His His 355 360 365

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<213> Bacillus licheniformis

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His Ala Gly Lys Asn Val Gln Ala Ala Glu Thr Tyr Glu Gln Leu Gln 50 60

Leu Leu Ala Asn Gln Tyr Thr Phe Glu Asp Glu Gln Trp Leu Thr Lys 65 70 75 80

Thr Ala Val Tyr Asp Ser Ala Glu Leu Lys Lys Glu Ile Gly Arg Leu 85 90 95

Thr Glu Cys Phe Pro Phe Val Thr Ser Arg Ile Ile Gly Arg Ser Ser 100 105 110

Met Gly Gln Pro Ile Tyr Glu Leu Leu Gly Ala Glu Asn Ala Gly 115 120 125

Lys Arg Thr His Met Asn Ala Ser Phe His Ala Asn Glu Trp Ile Thr 130 140

Thr Ser Val Leu Met Lys Trp Leu Lys Glu Tyr Cys Tyr His Leu Cys 145 150 155 160

Thr Gly Gln Thr Ala Leu Gly Phe Ser Pro Leu Asp Ile Phe Ser Ser 170 175 Page 191

Thr Lys Leu Ser Val Val Pro Ile Val Asn Pro Asp Gly Val Asp Leu 180 185 190 Val Leu Asn Gly Pro Gly His Leu Gly Ile Ala Arg Glu Ala Leu Asp 195 200 205 Glu Met Asn Glu His Gln Pro Asp Phe Arg Glu Trp Lys Ala Asn Ile 210 220 Asn Gly Val Asp Leu Asn Asn Gln Phe Pro Ser Phe Trp Glu Ile Glu 225 230 235 Lys Gln Arg Lys Pro Pro Lys Ser Pro Ser Tyr Arg Asp Tyr Pro Gly 245 250 255 Asp Glu Pro Leu Thr Glu Pro Glu Ala Ala Ala Met Arg Asp Leu Ile 260 265 270 Ala Asn Glu Pro Pro Asp Arg Leu Val Ala Leu His Thr Gln Gly Glu 275 280 285 Glu Ile Tyr Trp Gly Tyr Lys Gly Leu Glu Pro Pro Glu Ser Ala Asp 290 295 300 Val Ile Gln Thr Phe Glu Arg Leu Ser Gly Tyr Lys Gly Val Arg Tyr 305 310 315 320 Ile Asp Ser Tyr Ala Gly Phe Arg Asp Trp Phe Ile His Tyr Tyr Gly Arg Glu Gly Tyr Thr Val Glu Leu Gly Lys Gly Lys Asn Pro Leu Pro 340 345 Leu Lys Gln Phe Asp Asp Ile Tyr Cys Lys Ser Arg Gly Ile Leu Trp 355 360 365 Ala Ser Cys Phe Phe Glu Ser 370

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Arg Ala Lys Thr Tyr Asn Val Pro Lys Ala Ile Ile Glu Arg Ala Ile 65 70 80

Glu Lys Ala Lys Gly Gly Ser Glu Glu Asn Tyr Asp Glu Leu Arg Tyr 85 90 95 Page 194

Glu Gly Phe Gly Pro Asn Gly Ala Met Val Ile Val Asp Ala Leu Thr Asn Asn Val Asn Arg Thr Ala Ala Asp Val Arg Ser Thr Phe Gly Lys Asn Gly Gly Asn Met Gly Val Ser Gly Ser Val Ala Tyr Met Phe Asp Thr Ala Val Ile Gly Phe Glu Gly Lys Thr Ala Asp Glu Thr Leu 155 Ala Asp Asp Ala Val Ile Val Asp Ile Asp Val Arg Asp Ile Leu Gly Glu Ceu Leu Met Glo Ala Asp Ile Asp Val Arg Asp Ile Leu Gly Glu Gly Asp Asp Ala Val Ile Val Tyr Ala Gly Pro Asp Gln Phe His Ala Val Gln Glu Ala Leu Pro Glu Asp Ala Glu Ceu Thr Met Leu Ala Gln Asn Asp Val Ala Leu Pro Glu Asp Ala Arg Ala Arg Ala Gln Phe Glu Lys Leu Ile Asp Ala Leu Gly Asp Leu Glu Asp Val Gln Gln Val Tyr His Asn Val Asp Leu Gly Ala

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cag atc agg tct gct gtc gtc att ttc atc aca acc gca gtg ggc ggg Gln Ile Arg Ser Ala Val Val Ile Phe Ile Thr Thr Ala Val Gly Gly 420 425 430	1295
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Leu Gly Gly Thr Glu Asn Asn Gly Leu Thr Leu Glu His Val Val Tyr 35 40 45

Val Ile Arg Met Val Ser Leu Ala Leu Leu Val Val Pro Ile Leu Ala 50 . 60

Leu Ile Arg Gly Phe Phe Gln Gly His Gln Met Met Gly Pro Thr Ala 65 70 75

val Ser Gln Val Val Glu Gln Ile Ala Arg Ile Val Phe Leu Leu Thr 85 90

Ala Thr Tyr Leu Val Ile Lys Val Leu Asn Gly Gly Leu Val Val Ala 100 105 110

Val Gly Tyr Ala Thr Phe Ala Ala Leu Ile Gly Ala Phe Ala Gly Leu 125

Phe Thr Leu Tyr Phe Ser Trp Gln Lys Arg Lys Gly Ala Leu Leu Ala 130 135

Leu Lys Pro Asn Leu Val Pro Ser Ala Asp Ile Thr Tyr Arg Gln Met 145 150 160

Phe Lys Glu Leu Phe Ser Tyr Ala Ala Pro Tyr Val Phe Val Gly Leu 165 170

Ala Ile Pro Leu Tyr Gln Tyr Ile Asp Thr Asn Thr Phe Asn Lys Ala 180 185

Met Ile Ala Ala Gly Tyr Gln Asn Ile Ser Gln Asp Leu Met Ala Ile 200 205

Val Thr Leu Tyr Val Pro Lys Leu Val Met Ile Pro Val Ser Leu Ala 210 225

Thr Ala Phe Gly Leu Thr Leu Ile Pro Ala Val Thr Glu Asn Phe Thr 225 230 235

Asn Lys Asp Phe Pro Ala Leu Asn Lys Gln Ile Asp Gln Ala Met Gln 250 255 Page 198

Ile Ile Leu Phe Ile Val Leu Pro Ala Ser Val Gly Met Ala Leu Leu 260 265 Ser Gly Pro Val Tyr Thr Phe Phe Tyr Gly Ser Glu Ser Leu Leu Pro 275 280 285 Asp Met Gly Arg Asp Ile Leu Phe Trp Tyr Ala Pro Val Ala Leu Leu 290 300 Phe Ser Leu Phe Thr Val Asn Ala Ala Ile Leu Gln Gly Val Asn Lys 315 Gln Lys Phe Ala Val Val Ser Leu Met Ile Gly Ile Val Ile Lys Ile 325 Ala Leu Asn Val Pro Leu Ile Lys Leu Leu Gln Gly Ser Gly Ser Ile 340 345 Leu Ala Thr Ala Leu Gly Tyr Ser Ala Ser Leu Leu Tyr Gly Phe Ile 355 360 365 Met Ile Lys Arg His Ala Gly Tyr Ser Tyr Arg Lys Leu Phe Lys Arg 370 380 Phe Leu Leu Met Leu Ile Leu Thr Ala Val Met Gly Ile Ile Leu Leu 385 390 395 Leu Val Gln Ala Leu Leu Ser Ile Phe Ile Ser Tyr Glu Gly Gly Gln 405 410 Ile Arg Ser Ala Val Val Ile Phe Ile Thr Thr Ala Val Gly Gly Ser 420 425 430

Val Tyr Leu Tyr Leu Ala Tyr Arg Val Lys Leu Leu Glu Lys Ile Phe 435 440

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gaa gag gtg cag gaa gcg atc ttg aaa aaa gaa att tca gag cgc cac Page 200	1061

Glu Glu Val Gln Glu Ala Ile Leu Lys Lys Glu Ile Ser Glu Arg His 175	
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Bacillus licheniformis <213>

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Ile Pro Val Gly Asp Ile Ile Pro Asn Arg Phe Gln Pro Arg Thr Ile 35 40 45Page 201

Phe Ser Glu Glu Lys Ile Lys Glu Leu Ala Ala Thr Ile His Thr His 50 60 Gly Ile Ile Gln Pro Ile Val Val Arg Lys Thr Glu Arg Glu Gly Gln 65 70 75 Tyr Glu Leu Ile Ala Gly Glu Arg Arg Trp Arg Ala Val Gln Thr Leu 85 90 95 Asp Trp Glu Lys Val Pro Ala Ile Ile Lys Asp Phe Ser Asp Thr Glu 100 105 Thr Ala Ser Val Ala Leu Ile Glu Asn Leu Gln Arg Glu Glu Leu Ser 115 120 125 Ser Ile Glu Glu Ala His Ala Tyr Ala Arg Leu Leu Glu Leu His Asp 130 135 140 Leu Thr Gln Glu Ala Leu Ala Gln Arg Leu Gly Lys Gly Gln Ser Thr 145 150 155 160 Ile Ala Asn Lys Leu Arg Leu Leu Lys Leu Pro Glu Glu Val Gln Glu 165 170 175 Ala Ile Leu Lys Lys Glu Ile Ser Glu Arg His Ala Arg Ala Leu Ile 180 185 190 Pro Leu Lys Gln Pro Asp Leu Gln Val Lys Leu Leu His Glu Val Ile 195 200 205 Glu Lys Ser Leu Asn Val Lys Gln Thr Glu Asp Arg Val Val Lys Met 210 215 220 Leu Glu Gln Asp Lys Arg Lys Pro Lys Pro Lys Arg Lys Ala Tyr Ser 225 230 235 240 Arg Asp Ala Arg Ile Ala Met Asn Thr Ile Arg Gln Ser Leu Ser Met 255 Val Glu Asp Ser Gly Val Lys Leu Asm Thr Glu Glu Glu Glu Phe Glu 260 265 270 Glu Tyr Ile Gln Phe Thr Ile Arg Ile Pro Lys 275